Nguyet Phan
01/02/04 07:02 AM

To: NCIC HPV
cc:
Subject: HPV Submission CASNO 111-96-6

201-15023

Nguyet Phan
ASRC Aerospace
OPPT Docket
EPA Docket Center

----- Forwarded by Nguyet Phan/DC/USEPA/US on 01/02/04 07:03 AM -----

EJ Rauckman <erauckman@charter.net >
12/31/03 08:31 PM

To: NCIC OPPT@EPA, Rtk Chem@EPA
cc: Barry Misquitta <MisquittaB@Ferro.com>
Subject: HPV Submission CASNO 111-96-6

Michael O. Leavitt, Administrator

U. S. Environmental Protection Agency

P.O.Box 147

Merrifield, VA 22116

ATTN: HPV Challenge Program

Dear Administrator Leavitt

On behalf of Ferro Corporation (HPV registration number), I am submitting the attached test plan and robust summaries for Diglyme, CAS Number 111-96-6, submitted under the United States Environmental Protection Agency's High Production Volume Chemical Challenge Program.

This document is being submitted in electronic format (Adobe Acrobat pdf file). If you require additional information or have problems with the electronic document please contact me by phone (618-539-5280) or email (erauckman@charter.net).

Sincerely,

Elmer Rauckman PhD, DABT

Consulting Toxicologist for Ferro Corporation


Diglyme Test Plan.pdf


Diglyme Robust Sum.pdf

201-15023

Diglyme

CAS Number 111-96-6

$$Me\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}Me$$

U.S. EPA HPV Challenge Program Submission

29 December 2003

Submitted by:

Ferro Corporation
1000 Lakeside Avenue
PO Box 147000
Cleveland, OH 44114

Prepared by:
Toxicology and Regulatory Affairs
1201 Anise Court
Freeburg, IL 62243
618-539-5280

Table of Contents

Executive Overview

Diglyme, CAS no. 111-96-6, is an inert ethereal organic solvent that is not known to occur in nature. It is synthesized from ethylene oxide and methanol with production of over a million pounds per annum in the United States. It is a clear, water-like liquid with a mild "aromatic" odor. Diglyme is of relatively low volatility for an organic solvent with a vapor pressure of 3.49 hPa @ 25˚C. It has a freezing point of -68°C and a boiling point of 162°C. and is miscible with water and most organic solvents. Because of its excellent solvating properties, its most extensive use is as a solvent for applications where an inert solvent is required or where its superior solvent properties are an important consideration.

In the environment, based on physicochemical properties and experimental data, diglyme will not bioaccumulate (log $K_{o/w}$ = - 0.36) and will distribute primarily to water and secondarily to soil where it will be subject to volatilization and slow biodegradation under conditions favorable to bacteria. It is stable to hydrolysis but expected to react rapidly with atmospheric hydroxyl radicals with a half-life of about 7 hours. Diglyme is relatively nontoxic to aquatic species, with an acute LC_{50} for freshwater fish greater than 2000 mg/L, an EC_{50} for daphnia of greater than 1000 mg/L and an EC_{10} for green algae greater than 1000 mg/L. Diglyme is considered inherently biodegradable in the environment but is suffiently resistant to biodegradation that a significant portion of it may will be released unchanged from a typical waste water plant. The primary fate of diglyme in the environment is volatilization to the atmosphere were it is rapidly photo-degraded and aerobic biodegradation in water and soil with a half-life estimated as several weeks. Even with the limited biodegradation rate it presents little environmental hazard due to low toxicity to aquatic organisms.

The acute oral LD_{50} of diglyme is very high with a value of about 5000 mg/kg being reported for rat gavage studies. Exposure of rats to saturated vapor for 7 hours did not produce any significant adverse effects that were macroscopically visible at necropsy. The dermal LD_{50} in experimental animals in unknown but based on human skin absorption studies and a "read across" approach using analogs is expected to be greater than 2000 mg/kg in the rabbit. As with most glycol ethers, dermal absorption is viewed as a potentially significant route of exposure.

Repeated-dose studies by the oral or inhalation route; demonstrate that the male reproductive organs followed by the bone marrow are important target organs for high-level diglyme exposures. Although low-level exposures are well tolerated, evidence in experimental animals indicates the potential for serious adverse effects in with overexposure. Metabolic studies in animals indicate that 2-methoxyacetic acid is a minor but variable metabolic product of diglyme. As 2-methoxyacetic acid is considered to interfere with cellular proliferation, tissues with rapidly proliferation are both predicted to be and are in fact target organs in experimental animals. These tissues include the testes (sperm production) the bone marrow (blood cell production) and, in pregnant experimental animals, the developing conceptus.

Adequate *in vitro* tests of genetic toxicity for diglyme are available. Multiple *Salmonella typhimurium* reverse mutation assays show lack of mutagenic activity in the presence or absence of metabolic activation and *in vitro* DNA damage and chromosome aberration studies have produce negative results. A study investigating the in

vivo genotoxicity of diglyme after inhalation exposure indicated a lack of genotoxic activity as evidenced by no increase in bone-marrow cell chromosome aberrations after exposures to levels of diglyme that cause testicular damage.

Developmental toxicity has been investigated in rats using inhalation as the route of exposure, and investigated in mice and rabbits using oral administration. The rat and mouse study provided acceptable evidence o specific developmental toxicity from exposure of animals to diglyme. The rabbit study is less clear since the maternal NOAEL is stated as being lower than the developmental NOAEL; nevertheless, the effect on concepti are of sufficient magnitude to indicate specific developmental toxicity in the face of adverse maternal effects

The combination of developmental toxicity findings in treated pregnant animals and testicular toxicity in repeated dose studies indicates the potential of finding adverse reproductive effects. There is additional evidence that treated and affected males lose actual reproductive capacity. This evidence comes from a study designed to investigate if diglyme produced a dominant lethal effect. In this study, groups of male rats were exposed to diglyme vapor for five consecutive days and then were mated weekly without further treatment. High-dose treated males were unable to produce many offspring from weekly matings between week 5 and 9 after exposure. This is indicative of a male-mediated adverse reproductive effect of diglyme in experimental animals

It is concluded that the available information adequately fills all the data elements of the HPV. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, they provide adequate to meet all the screening data recommendations. Conduct of additional screening studies would not add significantly to our understanding of this material's hazard. Although studies beyond the screening set could provide valuable information relating to human hazard and risk assessment, such studies are beyond the scope of the current evaluation and no new HPV screening studies are recommended.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 111-96-6 Diglyme	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	Y	N	Y	N	
Boiling Point	Y	N	N	Y	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	N	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	N	N	Y	N	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	Y	N	Y	N	Y	N	
Ecotoxicity								
Acute Fish	Y	N	Y	Y	N	Y	N	
Acute Invertebrate	Y	Y	Y	Y	N	Y	N	
Acute Algae	Y	Y	Y	Y	N	Y	N	
Toxicity								
Acute	Y	N	Y	Y	N	Y	N	
Repeated Dose	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *"in vitro"*	Y	N	Y	Y	N	Y	N	
Genetic Toxicology "*in vivo*"	Y	N	N	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

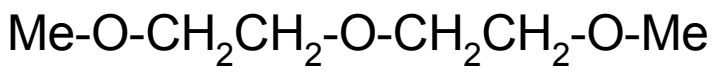
Introduction

Diglyme, CAS no 111-96-6, is a linear aliphatic diether that is a clear liquid at room temperature with what is described as a mild odor (1). It is miscible with water and organic solvents (2) and, because of its chemical inertness and excellent solvent properties, finds use as a specialty solvent for a wide variety array of applications. Diglyme is used as a reaction solvent for Grignard-reactions, reduction-reactions, alkylation-reactions, and organo metallic reactions in general. It finds application in reactions involving alkali metals such as lithium, sodium and potassium and can dissolve Na/K alloy, but potassium is only sparingly soluble. It dissolves vinyl chloride copolymers, polymethacrylate, polystyrene, polychloroprene and cellulose acetate (3). Other applications include in the coating industry and in photolithography for manufacture of semiconductor chips.

Diglyme is most commonly produced by reacting ethylene oxide with methanol in the presence of either acidic or basic catalysts although it can be made from diethylene glycol and dimethyl sulfate (HSDB). Occupational exposure during the manufacturing process is minimal as the production is in a closed system that is designed to provide adequate protection from the reactant ethylene oxide, which is a regulated chemical. Significant worker exposure is only possible during sampling, drumming and equipment maintenance.

No ACGIH TLV or other regulatory limit was located for diglyme. NIOSH, however, recommends reducing exposure for all glycol ethers to the lowest feasible concentration and preventing contact with the skin. (4).

Diglyme's structure is shown below:



Diglyme

Diglyme is also known as (5):

- Ethane, 1,1'-oxybis[2-methoxy- (9CI)
- Ether, bis(2-methoxyethyl) (8CI)
- Bis(2-methoxyethyl) ether
- Diethylene glycol dimethyl ether
- Diethyl Glycol Dimethyl Ether
- Diglycol methyl ether
- Diglyme
- 1,2-Dimethoxyethane
- Di(2-Methoxyethyl) ether

- Dimethyl carbitol
- Ethanol, 2,2'-oxybis-, dimethyl ether
- Ethylene glycol dimethyl ether
- Glyme-2
- 2-(2-Methoxyethoxy)-1-methoxyethane
- (2-Methoxyethyl) ether
- Methyldiglyme
- 1,1'-Oxybis(2-methoxyethane)
- Poly-Solv
- Poly-Solv D2M
- 2,5,8-Trioxanonane

Several physicochemical, fate and toxicity studies have been conducted on diglyme. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. The majority of data elements are filled by high-reliability studies on diglyme. Where direct data are not available or data are sparse, surrogates or estimation methods are used to fill the data element. The U.S. EPA and other regulatory authorities encourage this approach, where scientifically defensible, to avoid unnecessary testing and animal usage.

Physicochemical Data

Physicochemical data for Diglyme are available from the literature.

Table 1: Physicochemical Properties of Diglyme

Melting Point	-68° C (2)
Boiling Point	162° C @ 1010 hPa (2)
Vapor Pressure	3.49 hPa @ 25˚ C (6) (2.96mm)
Partition Coefficient	Log $K_{o/w}$ = -0.36 (7)
Water Solubility	Miscible (2)

These properties indicate that at ambient temperatures, Diglyme is a slightly volatile liquid with unlimited water solubility. The value of the partition coefficient suggests that Diglyme will partition preferentially into water;

therefore, only on the basis of the octanol-water partition coefficient, Diglyme is considered to have little potential for bioaccumulation.

Molecular Formula	C6-H14-O3
Molecular Weight	134.17
SMILES Code	COCCOCCOC

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Multiple investigations have been conducted on the biodegradation of diglyme and the data consistently indicate that diglyme can be aerobically biodegraded but is resistant to biodegradation in the usual screening tests. Tessier reported, in 1983, that highly oxygenated compounds, including diglyme, are difficult to degrade and when biodegradation does occur, it occurs slowly (8). Grant chemical (now Ferro) supported a study of the biodegradation of 1,4-dioxane and diglyme by adapted microorganisms. Results of this study, published by Roy et al (9) indicted that there was a population of bacteria in the waste treatment plant that could biodegrade diglyme but they apparently prefer other organic substrates to diglyme. Using a seed of dioxane/diglyme-adapted bacteria, pure diglyme, after a seven-day lag phase, was only partially (36%) degraded in 25 days of incubation. Diglyme in mixtures of dioxane and diglyme that were inoculated with these adapted bacterial was degraded by 85% in the presence of dioxane. The diglyme degrading bacterial population was also found to be inhibited by salt concentrations of one percent and greater. It was also postulated that dioxane is metabolized by these bacteria to a toxic product that inhibited further biodegradation when dioxane concentrations were suffiently high. Overall, these studies show that diglyme is biodegradable but is a poor substrate for even adapted bacteria. They also indicate the importance of a mixed population of bacteria and substrates to achieve optimal biodegradation.

Cowan and Kwon (10) extended the idea of adapting bacteria to mixed cultures of glycol ethers and other difficult to biodegrade ethers. They started with a bacterial culture from a petroleum refinery wastewater treatment plant and established these bacteria in a 5-liter Submerged Attached Growth Air Lift reactor (where the bacteria adhere to a fibrous support media and have an essentially infinite residence time). After 34 weeks of operation and optimization, the reactor effectively removed five of ten glycol ethers in the mixture, in the best case the diglyme removal by the adapted bacteria about 40%. This removal is better than that for four other glycol ethers that were only removed between 10 and 25% under optimized conditions this information allows a rough rank ordering of glycol ether's resistance to biodegradation which indicates that diglyme should be considered difficult to biodegrade, but not recalcitrant.

This classification is supported by two OECD guideline studies, one conducted by Hoechst AG and reported by IPCS (11) and the other by the European Chemicals Bureau (12). In the first test, a closed bottle test according to

OECD 301D, only 0.1% degradation (measured as oxygen uptake) was recorded in the first days of exposure and no 28-day degradation is reported (13). The second study was a modified Zahn-Wellens test in which the results are reported as 31% biodegradation after 28 days in the ECB IUDLID-2000 document and as 42% after 28 days by IPCS in the 2002 CICAD document (14).

Grossmann (15) recently described studies on chemically assisted decomposition of diglyme prior to introduction in a waste treatment plant that effectively utilized hydrogen peroxide and ozone based advanced oxidation processes to allow mineralization of diglyme. Fenton, photo-assisted Fenton and UV/H_2O_2 oxidation processes all showed acceptable TOC removals.

Photodegradation of chemicals can occur by two major mechanisms; direct, where the molecule has a chromophore that absorbs light in the range of wavelengths that impinge on the earth's surface; and indirect, where the chemical reacts with an atmospherically generated sensitizer such as hydroxyl radical or ozone. As diglyme has no chromophore that absorbs light in the appropriate range, direct photolysis is considered unimportant as a mechanism of photolysis. Indirect photolysis by reaction with atmospheric hydroxyl radical is anticipated based on diglyme's chemical structure.

Indirect photolysis was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 29.7 E-12 cm^3/molecule-sec (see accompanying robust summary in Appendix for full details); however, the SRC database contained an experimental value recorded by Dagaut and reported by Atkinson of 17.5 E-12 cm^3/molecule-sec (16). This experimental value is about half the value calculated by AOPWIN based on the chemical structure but both still predict a relatively short atmospheric half-life. The model predicted half-life, assuming a concentration of 1,500,000 hydroxyl radicals per cubic centimeter, is 4.3 hours. Using the experimental rate constant, the estimated atmospheric half-life is 7.33 hours. Given the preference for experimental data over calculated, and that the half-life derived from the experimental data is a more conservative number, the 7.3 hour atmospheric half-life for diglyme is accepted and has been applied in the EQC Level III modeling of environmental distribution.

Although water stability has not been quantitatively determined for diglyme in an OECD 111 guideline study, the National Toxicology Program has conducted a dosing vehicle test of aqueous diglyme solutions and concluded that it is stable in aqueous solution for a period of 21 days (17). In addition, water stability studies are considered unnecessary for compounds containing only non-hydrolysable groups. There is no evidence available in the literature that diglyme is unstable in water and the structure is that of a simple aliphatic ether, which is a class of molecule considered to be water unreactive at environmental pH values. The half-life in water is thus estimated as

greater than one year. This assessment is confirmed by the review of Harris, who notes specifically that ethers as a class are non-hydrolysable (18). Additional evidence attesting to its stability under basic conditions comes from its application as a preferred solvent for chemical reactions that occur under extremely basic conditions (19).

Theoretical Distribution (Fugacity) of diglyme in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05 but using the measured vapor pressure of 2.96 mm Hg, the measured log $K_{o/w}$ of -0.36, measured melting point, experimentally based photolysis estimate and data derived estimates of biodegradation (20). The results for environmental distribution using a model calculated $K_{o/c}$ (adsorption coefficient based on organic carbon content) of 0.179 and equal initial distribution to air, water and soil are:

- Air 0.505 %
- Water 60.4 %
- Soil 38.9 %
- Sediment 0.113 %

Recommendation: No additional fate studies are recommended. The available data fill the HPV required elements.

Ecotoxicity

Acute fish, invertebrate and alga studies have all been conducted for diglyme. Results of these studies and the ECOSAR predicted values using the neutral organics model are given in the table below.

Table 2: Comparative Aquatic Toxicity of Diglyme

	Reported Experimental Values	ECOSAR Prediction
Fish, 96 hour LC_{50} (DIN Guideline study)	> 2000 mg/L (21)	16,446 mg/L*
Daphnia, 48 hour EC_{50} (OECD Guideline study)	> 1000 mg/L (22)	14,971 mg/L*
Alga, 72 hour EC_{10} (OECD Guideline study)	> 1000 mg/L (23)	8,171 mg/L*

* Estimated using ECOSAR (24)

All of these studies have been conducted in accord with an appropriate guideline but the results are only available in the ECB IUCLID-2000 document (fish) or the IPCS CICAD document (daphnids and green algae) for diglyme. Robust summaries have been prepared giving what information is available to the public about these studies. Without the original reports to review, many details cannot be checked; however, the extensive international review by experts in ecotoxicity that the CICAD document receives prior to publication assures that there is established consensus surrounding these data. Other factors that were taken into considerations in recommending these studies be accepted for the EPA HPV program are that 1) with a highly water soluble, low volatility and stable material such as this, details of study conduct and verification of test substance are of less importance. 2.) Numerous materials of related structures have been evaluates in acute aquatic toxicity screening tests and have shown minimal toxicity to aquatic species. 3.) Quantitative SAR modeling (ECOSAR) predicted values for adverse effect levels from diglyme exposure are exceeding high and this QSAR model is typically accurate in predicting toxicity values for non-reactive and difficult to activate materials such as diglyme. Thus, although it is recommended that these data be accepted as fulfilling the requirements of the U.S. EPA HPV program, they are assigned reliability scores of 2 due to only being available in the secondary literature.

Recommendation: The fish, invertebrate and algal tests are adequate for the purposes of the HPV program screening evaluation. No additional aquatic screening studies are recommended.

Absorption, Distribution, Metabolism and Elimination

Absorption:

Multiple studies on the metabolism of diglyme in experimental animals demonstrate that diglyme is rapidly and completely absorbed from the gastrointestinal tract of the rat (28, 29) and the mouse (25). Toxicity following inhalation exposure confirms that diglyme is absorbed from the lungs, especially in the studies employing nose-only exposure (vide post). Dermal absorption has been shown in vitro using human skin and actually measuring the rate of movement across human skin (38). These results suggest that diglyme will be absorbed through the skin in vivo. The physicochemical properties of diglyme ($K_{o/w}$, neutral charge, molecular mass and excellent solvating properties) are also consistent with a material that will be absorbed by all routes of exposure.

Metabolism:

The major pathways for metabolism of diglyme have been identified in the rat and an understanding of this metabolism is helpful in understanding the toxicity of diglyme to experimental animals and man. Figure 1 indicates there are two initial metabolic oxidations that occur, with both implicated to involve Cytochrome P-450. The first, labeled as "Path A" is an oxidative dealkylation of an interior ether bond to formally give two molecules of 2-methoxyethanol; which has been extensively investigated with regard to its metabolism and toxicity. 2-Methoxyethanol is oxidatively converted, by way of the aldehyde, to 2-methoxyacetic acid (shown on the figure as bolded). 2-Methoxyacetic has been associated with testicular toxicity in male experimental animals and development of the conceptus in pregnant female animals In rats, most of the 2-methoxyacetic acid is excreted in the urine but some is conjugated with glycine to produce the amide N-methoxyacetyl glycine.

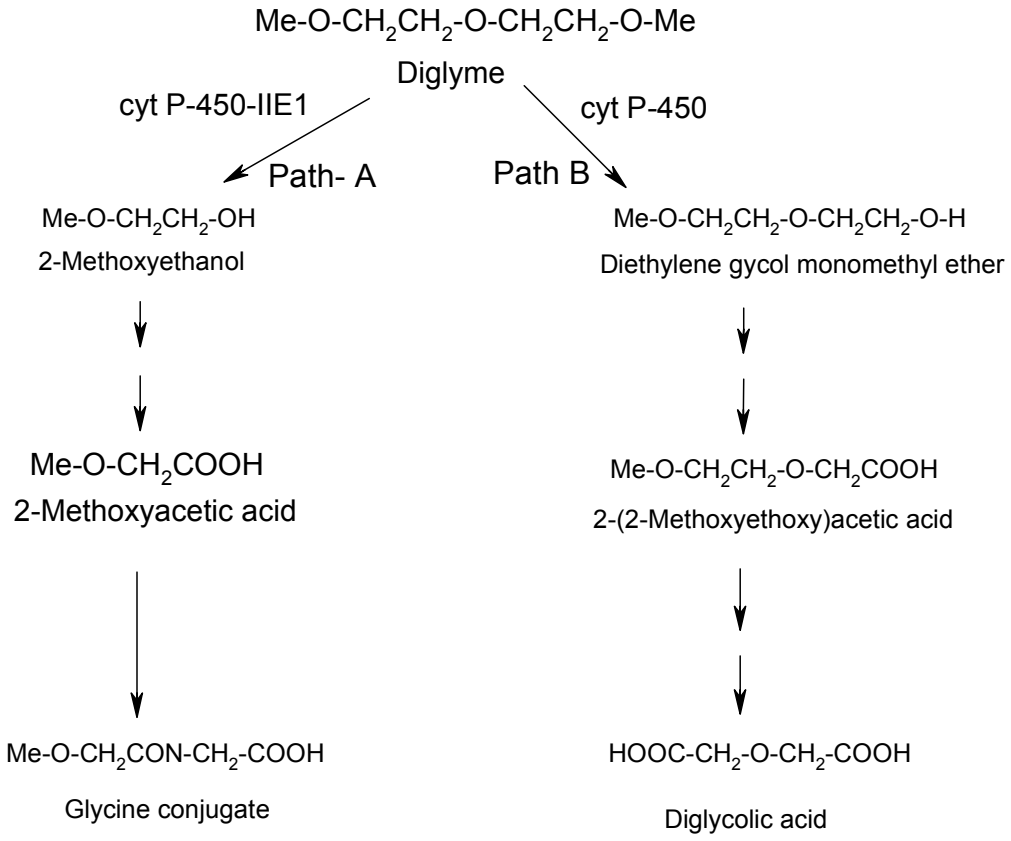


Figure 1: Metabolic Pathways for Diglyme in the Rat

Pathway B, involves oxidative demethylation of diglyme, by unspecified cytochrome p-450 isozymes to give 2-(2-methoxyethoxy)ethanol; which is oxidatively converted, by way of the corresponding aldehyde, to 2-(2-methoxyethoxy)acetic acid. Another oxidative demethylation on the other end of this molecule gives the alcohol 2-hydroxyethoxyacetic acid (not shown) that will be oxidized, through the corresponding aldehyde to the diglycolic acid and excreted.

Human and rat liver microsomal preparations have been shown to produce qualitatively and quantitively similar oxidative metabolic products suggesting that the human pathways for diglyme may be similar to those observed in experimental animals (26). In another report, it was stated that human-liver microsomes are even more efficient than rat-liver microsomes at cleaving diglyme into 2-methoxyethanol (27). Cytochrome P-450 induction by Phenobarbital, and to a lesser extent by diglyme itself, has been reported to increase the relative amount of diglyme that is metabolized to 2-methoxyethanol (28).

Studies in which rats were dosed with 2-(2-methoxyethoxy)ethanol or 2-(2-methoxyethoxy)acetic acid have been conducted and indicate that there is no "cross over" from the 2-(2-methoxyethoxy)ethanol pathway to produce the toxic metabolite 2-methoxyacetic acid (29). This indicates that which metabolic pathway diglyme will follow is primarily depended on the initial oxidative attach on the diglyme molecule. The initial pathway of attack is strongly influenced by the relative quantities of the various cytochrome P-450 isozymes that are present at any given time. Although no studies of human diglyme metabolism were found in the open literature, based on what is known about the metabolism of diglyme (vide supra) and what is known about the metabolism of glycol ethers in general (30), experimental results in animals are considered relevant to human hazard and risk assessment.

Health Effects

Acute Toxicity

Oral Exposure

Two acute oral studies on diglyme have been conducted on rats and one conducted on mice. These are shown in the following table.

Oral LD_{50} (mg/kg)	Species	Sex/strain	Comment	Reference
ALD = 7500	Rat	CD/male	Robust summary	31
4760	Rat	Wistar/female	From IUCLID-2000	32
2978	Mouse	CD-1/female	Published 1985	33

Table 3. Acute Oral Toxicity of Diglyme

The first study in the table is an "approximate lethal dose" (ALD) study conducted at eight dose levels using one animal per group. As the original report was available though NTIS, and the study has not been reviewed in any previous publication, a robust summary was prepared for this study and is included in the appendix. Due to the low statistical power of the ALD test, it cannot be determined if there is an actual difference in sensitivity between CD-1 male rats and Wistar females. All studies indicate a low order or acute oral toxicity for diglyme and no study identified a target organ.

Inhalation Exposure

The only specific acute inhalation study located for diglyme is an "inhalation risk test" using air "saturated" with diglyme, in which Wistar rats of each sex were exposed to diglyme vapor for 7 hours (34, 35). After exposure, animals were maintained for a 14-day observation period after which they were sacrificed and subjected to a gross necropsy. All animals survived exposure and a 14-day observation period. No macroscopic findings were observed at necropsy 14 days after the exposure. Clinical signs were restlessness, narrowing of palpebral fissures, and irregular breathing in rats. Based on the vapor pressure the concentration could have been as high as 3800 ppm (21 mg/L) but the actual nominal concentration was reported as > 11 grams/m^3, this is about 2000 ppm (see accompanying robust summary).

This result is supported by the results of repeated-dose studies reported by McGregor et al. (36), who exposed CD rats for 7 hours a day for 5 days at a measured diglyme concentration of 1000 ppm without mortality. It is also supported by the work of Valentine et al. (37) who exposed groups of 20 male and 10 female rats to measured concentrations of up to 1100 ppm diglyme, six hours a day five days a week for 10 exposures without mortality.

Dermal Exposure

No acute dermal studies were located for diglyme; however, there is a recent measurement of the ability of diglyme to penetrate human skin relative to six other glycol ethers published by Filon et al. (38). Eight other glycol ethers were evaluated by Dugard et al. using a similar method (39). An estimate of the likely skin penetrating ability of diglyme can be derived by examination of Table 4.

Structure (H_2's Excluded)	Common name	Rate of absorption (mg/cm^2-hr)		Rabbit Dermal LD_{50}	Rat Oral LD_{50}
		Dugard et al (38)	Filon et al (39)		
Me-O-CC-OH	2-Methoxyethanol	2.82		1300 (40)	3250 (40)
Me-O-CC-O-CC-O-Me	Diglyme		0.952		4760
Et-O-CC-O-Ac	2-Ethoxyethanol acetate	0.800		1818 (41)	2900-7500 (52)
Et-O-CC-OH	2-Ethoxyethanol	0.796	0.820	3311 (40)	2125-5487 (52)
Et-O-CC-O-Et	Ethylene glycol diethoxy ether	0.125	0.166		>4390 (42)

Table 4. Measured Human Skin Absorption Rates of Some Glycol Ethers.

Keeping in mind that the 95% confidence interval surrounding each determined rate of absorption is large, it can be surmised that diglyme probably penetrates skin with about the same facility as 2-ethoxyethanol and 2-ethoxanol acetate, both of which have dermal LD_{50} values for the rabbit equal to or lower than the rat oral LD_{50} values. Without attempting to correlate intrinsic toxicity with skin and intestinal absorption and toxicokinetic considerations, a simple read across approach suggests that the rabbit dermal LD_{50} for diglyme is in the range of 2000 to 4000 mg/kg. As the current practice for conducting dermal toxicity testing limits the top dose to 2000 mg/kg (43), it is unlikely that any significant information would be obtained by the actual conduct of a dermal study.

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, the weight of evidence shows that the oral and inhalation acute toxicity is very low. Sufficient information about the dermal penetration of diglyme absorption though human skin is available to assess the hazard of dermal toxicity. Conduct of additional acute-toxicity screening studies studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Oral Exposure

The only repeated dose oral study found I the literature that was not focused on reproductive function is a study in which four male JCL-ICR mice were treated with diglyme in the drinking-water for 25 days at a level of 2% (44). At the end of the exposure period the number of total white blood cells was more than twice that of controls. Although this finding is of possible interest the study design lacked the power to produce a statistically significant effect. Repeated-dose studies on potential effects of diglyme on male reproductive are found in the reproductive toxicity section. Inhalation is considered the more relevant route.

Inhalation Exposure

A modern inhalation study with analytically determined concentrations with recovery groups and a positive control compound (2-methoxyethanol) has been reported by Valentine et al. (45). In this study, groups of 20 male and 10 female rats were exposed to 0, 110, 370, or 1100 ppm diglyme, six hours a day five days a week for 10 exposures. Male rats were killed after 10 days of exposure and 14, 42, or 84 days post-exposure. Female rats were killed after the 10th exposure and 14 days latter. Urine analysis, hematological analyses, clinical chemistry and histopathology were performed. Changes in the hematopoietic system occurred in males and females involving

bone marrow, spleen, thymus, leukocytes, and erythrocytes. The NOAEL for female rats was 370 ppm. Males were more sensitive than females and the primary target organ for males was found to be the reproductive system. Stage-specific germ cell damage occurred at all concentrations and was concentration and time dependent Effects on the male reproductive system at a concentration of 110 ppm were relatively mild and can be considered a LOAFL. The effects produced by 300 ppm 2-methoxyethanol were more severe than produced by 370 ppm diglyme but not as severe as produced by 1100 ppm diglyme under the same conditions of exposure. It was concluded that diglyme is one-half to one-third as potent as 2-methoxyethanol under the same inhalation conditions.

As a NOAEL for testicular effects was not identified, a second study using the same design was performed with lower concentrations of diglyme (46). In this study, measured concentrations of 0, 3.1, 9.9, 30, or 98 ppm were used to expose rats by inhalation for 10 exposures followed by a 14-day recovery period. Mean body weights of rats exposed to 98 ppm were significantly lower than those of controls at the end of the exposure period. The weights of testes, seminal vesicles, prostate, and epididymides were similar to controls. Microscopic examination of the testes revealed minimal or mild testicular atrophy in the 100-ppm group. Findings at lower concentrations were not considered to be compound related. It was concluded that the target organ is the male reproductive system and the NOEL for male rats is 30 ppm under these conditions.

Recommendation: No additional repeated-dose studies are recommended. The available data fill the HPV required endpoint for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate tests have been conducted that cover both of these endpoints. Complementary studies have also been conducted and the overall weight of evidence indicates lack of genotoxic potential for diglyme.

Genetic Toxicology in vitro

Results from the *Salmonella typhimurium* reverse mutation assay have been negative. The NTP conducted and reported tests using microsomal activation system from two different species (47). Their findings indicate that neither rat liver or hamster-liver metabolic activating systems induces diglyme to have any mutagenic activity in this test system. The results from the NTP studies (48) have been summarized robustly and appear in the Appendix. Other Salmonella typhimurium reverse mutation assays have been conducted and reported to be negative. McGregor reported two of these (49) and the other is contained in two unpublished reports from Hoechst AG (50, 51).

Another DNA damage test was conducted using human cells (49). In this study, human embryonic intestinal fibroblasts were observed for unscheduled DNA synthesis after 3 hours of in vitro exposure to up to 19mg/ml diglyme and did not give a response indicative of diglyme causing any damage to human DNA

Genetic Toxicology in vivo

Information from un-confounded genotoxicity studies conducted *in vivo* is limited to a report in which bone marrow cells form groups of 10 CD rats of each sex exposed to 250 or 1000 ppm diglyme for seven hours a day for 1 or 5 days were examined for chromosome aberrations (49). An increase in chromosome aberrations was not observed in bone marrow cells of either sex at either dose. This study is considered and adequate test of the clastogenic potential of diglyme because it was conducted by a scientifically defensible method and because the high dose level was documented to produce severe toxicity to the testes of male rats exposed under the same conditions as described in the *Fertility* section of this document (and the robust summery covering fertility).

Other attempts at in vivo genotoxicity testing of diglyme were a recessive lethal test on *Drosophila melanogaster* that could not be evaluated because of an unusually high death rate in a control group and a dominant lethal test in rats (49). The rat study showed a reduced number of pregnancies and an increase in preimplantation losses but it could not be determined if this was due to a dominant lethal effect or to reduced fertility of the males. In light of the established adverse effects of diglyme on fertility, it was assumed that reduced fertility was the cause and not a dominant lethal effect. This is considered a reasonable assumption as diglyme showed no genotoxic activity in other assays and as the class of compounds known as glycol ethers is considered to have low potential for inducing genetic damage (52).

Recommendation: The SIDS requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using acceptable protocols. No additional genotoxicity testing is recommended.

Reproductive Toxicity

The combination of the positive developmental toxicity studies in rats and mice with repeated dose studies (see repeated dose section and accompanying robust summaries) showing that the reproductive organs of male experimental animals is a specific target organ for diglyme, is sufficient information to fulfill the relevant requirement for reproductive toxicity information. In addition to this information, a dominant lethal test in rats is available that provides definitive information about the effect of diglyme on male reproductive function and, also

located in the literature, was a specific study of the effect of diglyme on the testes of Sprague-Dawley rats (*vide infra*).

Cheever et al. (53) investigated the effect of repeated orally administered diglyme on the pathology and lactate-dehydrogenase-X (LDH-X) levels of the testes of Sprague-Dawley rats. Daily administration of diglyme at 5.1 millimole/kg body weight diglyme to male Sprague-Dawley-rats resulted in reduced body weight gain and reduced testis weights. The testis weights revealed reductions after as few as ten doses while reduction in body weight gain only occurred after 18 consecutive daily doses. The testes, epididymides, and thymus glands of rats given 20 doses of diglyme demonstrated significant decreases in relative weights. Degenerative changes were noted on histopathological examination of the testes after 20 consecutive days of dosing and degenerative changes were noted in the germinal cells after as few as eight doses. Cessation of treatment resulted in some evidence of regeneration of spermatocytes beginning at 2 weeks after stopping treatment. Decreased LDH-X isoenzyme activity was noted in testis homogenates.

The dominant lethal test consisted of groups of 10 male adult CD rats exposed to 0, 250, or 1000 ppm diglyme for seven hours a day on five consecutive days, then serially mated at weekly intervals for 10 weeks to untreated virgin females (54, 49) (also see associated robust summary for this study). The presumed-pregnant female rats were killed and examined 17 days after they were first caged with the males. No effect on frequency of pregnancy was seen in the 250-ppm exposure group. Large reductions in pregnancy frequency, however, occurred in the 1000-ppm exposure group in weeks 4 through 9, especially in weeks 5 through 7 after exposure, when pregnancy frequencies were only about 10%. Most of the loss was attributable to preimplantation loss. Although transformation of the data allowed interpretation of the data that implied post-implantation loss in weeks 5 and 6, this conclusion was confounded by the know increase in post-implantations loss in dams where total implantation is low. In conclusion, the results of the test are equivocal regarding a dominant lethal effect and the authors concluded that the effect of diglyme "on male fertility and embryonic development are of much greater importance than genetic effects when setting tolerable limits"

The investigators focused on analysis of the data to investigate if there was a significant post-implantation loss as a test of genotoxicity, rather than looking on this primarily as a study of male fertility. Although it could not be ascertained if diglyme has any activity in causing a dominant lethal effect, the results of the study are very valuable in definitively demonstration a clear adverse effect of diglyme on functional male fertility. The design of the study also lends itself to indicating that the initial effects in the testes are primarily on the early stages of sperm development, probably the mitotic cells (spermatocytes) and not on the germinal epithelium or the haploid stages of the sperm, which were apparently unaffected in their ability to effectively cause pregnancy and viable fetuses. In addition, the study indicates that although the effects on sperm can be severe enough to restrict the induction of pregnancy, the effects are largely reversible in rats after 10 weeks of recovery (at least for males that are mated weekly).

Additional supporting information on the effect of diglyme on sperm comes from a "Sperm abnormality test in mice" reported by McGregor and co-workers (49) in which inhalation exposure of male mice to 1000 ppm

diglyme vapor for 7 hours a day for 4 consecutive days increased the incidence of sperm with "amorphous heads" from the control level of 2.2% to 20.9% upon examination 35 days after exposure. Other categories of sperm abnormalities were also significantly increased including "hook turned-up or elongated", "banana-shaped head" and "hair pin or tight coil tail". Exposure of mice to 250-ppm diglyme vapors for 7 hours/day for five days did not affect sperm morphology.

Recommendation: No additional reproductive testing is recommended, as the available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

Adequate developmental toxicity studies of diglyme have been conducted using rats, mice and rabbits. Diglyme has consistently produced results that indicate it is a specific developmental toxin in experimental animals. A total of five definitive or screening studies were located in the open literature and these are shown in Table 5 with the maternal and fetal NOAELs. The two mouse studies at the bottom of the table do not have entries for NOAELs because, with few dose levels and limited fetal examinations, they were not designed to identify a NOAEL but were designed simply to indicate the potential for hazard.

Species	Route	Notes	Dev Tox ?	**Maternal NOAEL**	**Fetal NOAEL**	**Reference**
rats	Inhalation	Used 2-methoxyethanol as a positive control	+	25 ppm (100 ppm)*	<25 ppm (25 ppm)*	DuPont 1988 (55); Driscoll et al. 1998 (56)
rabbits	gavage	Reassessed NOAEL in journal publication	+	25 mg/kg**	50 mg/kg**	NTP 1987 (57); Schwetz et al. 1992 (58)
mice	gavage	**Four-dose study**	**+**	500 mg/kg	62.5 mg/kg	NTP, 1985 (59): Price et al. 1987 (60)
mice	gavage	Limited fetal examination, Positive result	+			Hardin & Eisenmann,1987 (61)
mice	gavage	Chernoff-Kavlock test, Positive result	+			Schuler et al. 1984 (62)

* = Alternative interpretation of NOAEL

** = These NOAELs are controversial (see text below)

Table 5. Available Developmental Toxicity Studies of Diglyme

These positive finding in experimental animals are not surprising in light of the information about metabolism of a fraction of diglyme to 2-methoxyacetic acid in animal models. 2-Methoxyacetic acid has been implicated as the proximate developmental toxin of 2-methoxyethanol and its occurrence as a significant metabolite of diglyme links 2-methoxyethanol and diglyme through a common metabolite. It must be keep in mind, however, that 2-methoxyacetic acid is not a necessary metabolite of diglyme and its existence and relative quantity could vary across species and among individuals depending on genetic makeup and environmental conditions that affect enzyme expression. In addition to these metabolic aspects, risk to humans is dependent on exposure, other

pharmacokinetic determinants and pharmacodynamics factors; most of which have not been fully characterized. Further discussion of human risk assessment, however, is beyond the scope of this document.

The DuPont inhalation study used dose levels of 0, 25, 100 or 400 ppm diglyme as a vapor to expose pregnant rats using a "nose only" procedure where the rats were restrained in stainless steel cylinder during the six hours of exposure. The study also used a positive control substance, 2-methoxyethanol, at a concentration of 25 ppm (equimolar basis with 25 ppm diglyme but about half the amount on a weight basis). At an exposure concentration of 400 ppm, all litters were completely resorbed and maternal toxicity was manifest as reduced food consumption. Animals exposed to 100 ppm had increased liver weights as the manifestation of maternal toxicity. Malformations were found in low incidences at 25 ppm and 100 ppm and included abnormally formed tails, distended lateral ventricles of the brain, axial skeletal malformations (vertebral fusions, hemivertebrae), and appendicular malformations (aberrant clavicular and scapular formation, bent fibula, radius, tibia, and ulna). Structural variations, primarily delayed ossification, were found in both these groups. The lowest dose of 25 ppm (140 mg/m3) caused a slightly increased incidence of variations. Although these defects were not significantly different from control values (except for the incidence of skeletal developmental variations), the pattern, type, and incidence of variations were similar to those seen at the effect level of 100 ppm. The authors, based on this similarity, suggested that 25 ppm was an effect level that approaches the lower end of the developmental toxicity response curve. Therefore, 25 ppm was considered a LOAEL for developmental effects and a NOAEL for maternal effects.

The positive control group exposed to 25 ppm 2-methoxyethanol manifest maternal toxicity as decreased feed consumption and increased liver weights. It was noted by the authors that the incidence and severity in the diglyme and 2-methoxyethanol groups exposed to 25-ppm vapors was essentially the same suggesting similar potency for producing structural variations. This comparison, however, is confounded by the maternal toxicity reported for the 2-methoxyethanol exposed dams and by the unknown contribution of the added maternal stress of being tightly confined for 6-hours a day during the nose-only exposure. Another confounding factor in these conclusions is that fetuses from the 25-ppm diglyme exposed group were not statistically affected except for a slight increase in variations over the control group.

In light of the low exposure level calculated on a mg/kg basis for the putative 25 ppm LOAEL and the confounding factors listed above, a possible reinterpretation of the results to view the maternal liver weight gain as an adaptive change and not a manifestation of toxicity, accompanied by accepting the statistical interpretation that the 25 ppm diglyme exposed fetuses are not different from controls might be a reasonable alternative interpretation. In this case the maternal NOAEL becomes 100 ppm and the developmental NOAEL would be set at 25 ppm. This approach allows a definitive NOAEL, which is better for risk assessment purposes and brings the data more in line with data from other glycol ethers in the literature and the positive control findings. An additional factor that must be taken into consideration when comparing diglyme with 2-methoxyethanol is that on a ppm basis there is gravimetrically twice the dose of diglyme being delivered as 2-methoxyethanol and as pointed out in the metabolism section, one diglyme molecule has the potential to be converted into two 2-methoxyacetic acid molecules.

The rabbit gavage study was conducted by NTP using timed pregnant New Zealand White rabbits (15-22 dams per group) dosed by water gavage on gestational days (gd) 6 through 19 at doses of 0, 25, 50, 100 or 175 mg/kg-day (57, 58). Doses were selected based on the results of preliminary dose range-finding studies. Treated females were sacrificed on gestational day 30, uterine contents were inspected, and live fetuses examined for malformations.

Clear evidence of maternal toxicity was only observed at the 175 mg/kg-day dose level where mortality among treated females was 15.4% as compared to 4% among controls. Maternal toxicity may have occurred in all groups of diglyme treated animals as body weight gain during the treatment period in these groups was less than in controls; however, corrected body weigh gain of dams did not differ between groups. Because of this, the maternal NOALE is controversial (*vide post*).

At 50 mg/kg-day, apparent adverse effects on prenatal growth, viability and morphological development were in accord with significant dose-response relationships observed across all groups, bur no individual measure reached statistical significance. At dose levels of 100 or 175 mg/kg-day, adverse effects upon fetal weight were in agreement with significant dose-response relationships, but individual treatment groups did not differ significantly from controls. The incidence of resorptions and malformed live fetuses, as well as other cumulative indices which included these endpoints, were significantly above control incidence only at dose levels of 100 and 175 mg/kg-day. Major malformations included development of the digits, craniofacial structures, abdominal wall, cardiovascular system, urogenital organs and axial skeleton. The most frequently observed individual defects were fusion of ribs (19%), hydronephrosis (23%), and clubbing of the limbs (19%) without underlying bone deformities. The incidence of adverse developmental effects was increased at a dose associated with increased maternal mortality (175 mg/kg-day). The principal manifestations of developmental toxicity at this dose were increased resorptions and a higher incidence of major malformations among surviving fetuses.

The originally published NTP report of this study published in 1987 offered the 100-mg/kg dose level as the maternal NOAEL and the 25 mg/kg level as the developmental NOAEL (57). When published in a peer-reviewed journal in 1992, however, the data had been reinterpreted and the maternal NOAEL was restated as 25 mg/kg and the developmental NOAEL as 50 mg/kg (58). This reinterpretation changes the Adult/Developmental ratio (A/D ratio), a common metric in developmental toxicity hazard assessment, from a value of 4.0 to a value of 0.5; this is a change that typically represents a reversal in developmental hazard assessment. Furthermore, in a review of the diethylene and triethylene glycol ethers' reproductive and developmental effects in 1996, Kimmel states that the maternal and developmental NOAEL for diglyme in this rabbit study are both 25 mg/kg-day (63), referencing the 1992 publication (58) as the source of this information. These discrepancies are an example of the difficulty of determining a robust maternal NOAEL in the face of actual developmental toxicity affecting total body weight gain of the pregnant dams. In spite of these limitations, the study does provide information that the rabbit is a sensitive species and that the malformations reported did not affect an individual organ system but were more nonspecific and primarily associated with visceral and external malformations and not the skeletal system.

The two screening studies listed in the table provide additional supporting evidence that diglyme has potential to be a developmental toxin in mice of other strains (61, 62). Taken together, all five of these studies provide consistent and convincing evidence that diglyme is a developmental toxin in experimental animals. Additional studies on the metabolism of diglyme to 2-methoxyacetic acid in experimental animals strengthen this conclusion and provide a mechanistic basis for the developmental toxicity of diglyme.

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material relative to the requirements of the HPV program.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provides a reliable hazard assessment. Conduct of additional SIDS-screening studies would not add significantly to our understanding of this material's toxicity.

References

1 Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993 page 392

2 O'Neil, MJ (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001

3 Stoye, D. *Solvents* in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VHC article dated 15 June 2000.

4 NIOSH/CDC. NIOSH Recommendations for Occupational Safety and Health Standards 1988, Aug. 1988. (Suppl. to *Morbidity and Mortality Wkly.* Vol. 37 No. 5-7, Aug. 26, 1988). Atlanta, GA: National Institute for Occupational Safety and Health, CDC, 1988

5 Chemical Information System, NISC International, Inc. Baltimore, Maryland

6 Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989 as cited in National Library of Medicine Hazardous Substance Data Base, Record #72, Last Revision Date: 20030305

7 Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society. 1995. page 97

8 Tessier, S et al. Degradation of Polyoxyethylenes: Biodegradation using an Enzyme from Psuedomonas P-400 and Chemical Degradation using Sodium Hypochlorite. *J. Chem research* 1983: 174-175 (1983)

9 Roy, D. Anagnostu, G. Chaphalkar, P. Biodegradation of Dioxane and Diglyme in Industrial Waste. *Journal of Environmental Science and Health Part A Environmental Science And Engineering*; 29 (1). 1994. 129-147

10 Cowan, RM, et al. Activated Sludge and Other Aerobic Suspended Culture Processes Water Environment Research; 67 (4). 1995. 433-450

11 International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

12 European Chemicals Bureau, IUCLID 2000 for CASNO 111-96-6

13 Hoechst AG (1989): Unveroeffentlichte Untersuchung (V–89–903–A) as cited in European Chemicals Bureau, IUCLID 2000 for CASNO 111-96-6

14 Hoechst (1989) *Prüfberichte: ökologische Untersuchungen.* Frankfurt am Main, Hoechst AG, 4 pp. (unpublished report). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

15 Grossmann D, Koser H, Kretschmer R, Porobin M. Treatment of diglyme containing wastewater by advanced oxidation--process design and optimisation. *Water Sci Technol*. 44:287-93 (2001).

16 Dagaut P, Wallington IJ, Liu R, Kurylo MJ (1988) 22nd International Symposium on Combustion, Seattle, WA; as cited in Atkinson R (1989) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. Journal of Physical and Chemical Reference Data, 1:143. as cited in Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

17 Teratologic evaluation of diethylene glycol dimethyl ether (CAS No. 111-96-6) administered to New Zealand White rabbits on gestation days 6 through 19. (1987) Research Triangle Park, NC, National Institute of

Environmental Health Sciences, National Toxicology Program (NTP-87-108; PB 87-209532). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

18 J.C. Harris in Lyman W, Reehl, W and Rosenblat, D.(1990) Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C.

19 See http://www.glymes.com/diglyme.php for examples.

20 EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

21 Hoechst Abwasserbiologische Untersuchung von Dialkylglykoläthern auf die Goldorfe (Leuciscus idus). Report Nr.79.0555 (1979) as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002 and using data found in the ECB IUCLID 2000 document for diglyme.

22 Hoechst (1994) Prüfung der toxischen Wirkung von Diethylenglykoldimethylether auf Kleinkrebse (Daphnia magna). Hoechst AG, Frankfurt am Main (unpublished report) .as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

23 Hoechst (1995) Prüfung der Schadwirkung gegenüber Algen (Algentoxizität) von Diethylenglykoldimethylether. Hoechst AG, Frankfurt am Main (unpublished report). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

24 ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

25 Daniel FB, Cheever KL, Begley KB, Richards DE, Weigel WW, Eisenmann CJ Bis(2-methoxyethyl) ether: metabolism and embryonic disposition of a developmental toxicant in the pregnant CD-1 mouse. Fundam Appl Toxicol 16:567-75 (1991).

26 Tirmenstein MA Comparative metabolism of bis(2-methoxyethyl) ether by rat and human hepatic microsomes:Formation of 2-methoxyethanol. *Toxicology in vitro* ,7:645–652 (1993)

27 Toraason M, Richards DE, Tirmenstein MA (1996) Metabolism of diglyme by rat hepatocytes and human microsomes. *Occupational hygiene*, 2:33–43 (1996).

28 Cheever KL, Richards DE, Weigel WW, Begley KB The role of enzyme induction on metabolite formation of bis(2-methoxyethyl) ether in the rat. *Toxicology and industrial health* 5:601–607 (1989).

29 Cheever KL, Richards DE, Weigel WW, Lal JB, Dinsmore AM, Daniel FB (1988) Metabolism of bis(2-methoxyethyl) ether in the adult male rat: evaluation of the principal metabolite as a testicular toxicant. *Toxicology and applied pharmacology*, 94:150–159

30 The Toxicology of Glycol Ethers and its Relevance to Man. Tech Report No. 64 ECETOC (European Centre for Ecotoxicology and Toxicology of Chemical) Brussels, Belgium 1995.

31 DuPont Chemical, Haskell Laboratories report 51-73. Initial Submission: Acute Oral Test of Ether,Bis(2-Methoxyethyl) In Rats With Cover Letter Dated 101592. U.S. EPA/OPTS Public Files, Document Number: 88-920009811, TSCATS mfiche.

32 Hoechst AG Akute orale Toxizität von Diethylenglykoldimethylether an weiblichen Ratten. Frankfurt am Main, Hoechst AG, 4 pp. (Report 376/79; unpublished) (1979). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002 and data as found in ECB IUCLID 2000 document for diglyme.

33 Plasterer MR, Bradshaw WS, Booth GM, Carter MW, Schuler RL, Hardin BD. Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: naphthalene, p-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives. Journal of Toxicology and Environmental Health, 15:25–38 (1985).

34 Hoechst (1979a) *Inhalationstoxizität im Zeitsättigungstest von Diethylenglykol-dimethylether an männlichen und weiblichen SPF-Wistar-Ratten.* Frankfurt am Main, Hoechst AG, 10 pp . (Report 488/79; unpublished) as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

35 Hoechst AG : Unveroeffentlichte Untersuchung (79.0488) 1979 as cited in ECB IUCLID-2000 for diglyme.

36 McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. *Toxicology and Applied Pharmacology*, 70:303–316 (1983).

37 Valentine R, O'Neill AJ, Lee KP, Kennedy GL Jr. Subchronic inhalation toxicity of diglyme. *Food Chem Toxicol*. 37:75-86 (1999).

38 Larese Filon F, Fiorito A, Adami G, Barbieri P, Coceani N, Bussani R, Reisenhofer E. Skin absorption in vitro of glycol ethers. *Int Arch Occup Environ Health.* 72:480-4 (1999).

39 Dugard PH, Walker M, Mawdsley SJ, Scott RC. Absorption of some glycol ethers through human skin in vitro. *Environ Health Perspect.* 57:193-7 (1984)

40 Carpenter, CP et al. The Toxicity of Butyl Cellosolve Solvent. *AMA Arch Ind Health* 14:114 (1956).

41 Carpenter, CP Queries and Minor Notes. *J Am Med Assoc*. 135:880 (1947).

42 Symth, HF. Seaton, J and L. Fisher The Single Dose Toxicity of Some Glycols and Derivatives. *J Ind Hyg Toxicol*. 23:259-268 (1941).

43 OECD Dermal Acute Toxicity Guideline 402 (24 February 1987).

44 Nagano K, Nakayama E, Oobayashi H, Nishizawa T, Okuda H, Yamazaki K. Experimental Studies on Toxicity of Ethylene Glycol Alkyl Ethers in Japan. *Environmental Health Perspectives*, 57:75–84 (1984).

45 Valentine R, O'Neill AJ, Lee KP, Kennedy GL Jr. Subchronic inhalation toxicity of diglyme. *Food Chem Toxicol.* 37:75-86 (1999)

46 DuPont (1989) *Subchronic inhalation toxicity study with diglyme.* Newark, NJ, E.I. Du Pont de Nemours & Co., 187 pp . as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

47 Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environmental mutagenesis*, 8(S7):1–119 .

48 Data from this study is found on the NTP public database at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

49 McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW (1983) Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. Toxicology and Applied Pharmacology, 70:303–316.

50 Hoechst (1979) *Test for mutagenicity in bacteria strains in the absence and presence of a liver preparation*. Frankfurt am Main, Hoechst AG, 7 pp. (Report 53/79; unpublished). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

51 Hoechst (1979) *Mutagenicity evaluation of diethyleneglycoldimethylether in the Ames* Salmonella*/microsome plate test.* Frankfurt am Main, Hoechst AG, 15 pp. (Report 743/79, unpublished). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

52 ECETOC *The toxicology of glycol ethers and its relevance to man.* Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals, pp. 1–350 (Technical Report No . 64), 1995.

53 Cheever KL, et al. Testicular Effects of Bis(2-methoxyethyl) Ether in the Adult Male Rat Toxicology and Industrial Health, 5:1099-1109 (1989)

54 McGregor DB. Genotoxicity of Glycol Ethers Environmental Health Perspectives 57:97-103 (1984).

55 DuPont Haskell Laboratory *Teratogenicity study of diglyme in the rat* . Newark, NJ, E.I. Du Pont de Nemours & Co., 289 pp (1988).

56 Driscoll CD, Valentine R, Staples RE, Chromey NC, Kennedy GL Jr Developmental toxicity of diglyme by inhalation in the rat. *Drug and chemical toxicology*, 21(2):119–136 (1998).

57 NTP Teratologic evaluation of diethylene glycol dimethyl ether (CAS No. 111-96-6) administered to New Zealand White rabbits on gestation days 6 through 19. Research Triangle Park, NC, National Institute of Environmental Health Sciences, National Toxicology Program NTP-87-108; PB 87-209532, (1987)

58 Schwetz BA, Price CJ, George JD, Kimmel CA, Morrissey RE, Marr MC The developmental toxicity of diethylene and triethylene glycol dimethyl ethers in rabbits. *Fundamental and applied toxicology*, 19(2):238–245 (1992).

59 Teratologic Evaluation of Diethylene Glycol Dimethyl Ether (CAS NO. 111-96-6) Administered to CD-1® Mice on Gestational Days 6 through 15. NTP Study TER85061, Report Date: August 30, 1985 NTIS#PB86-135233

60 Price CJ, Kimmel CA, George JD, Marr MC. The developmental toxicity of diethylene glycol dimethyl ether in mice. *Fundamental and applied toxicology*, 8:115–126 (1987).

61 Hardin BD, Eisenmann CJ (1987) Relative potency of four ethylene glycol ethers for induction of paw malformations in the CD-1 mouse. *Teratology*, 35:321–328

62 Schuler RL, Hardin BD, Niemeier RW, Booth G, Hazelden K, Piccirillo V, Smith K (1984) Results of testing fifteen glycol ethers in a short-term *in vivo* reproductive toxicity assay . *Environmental health perspectives*, 57:141–146 .

63 Kimmel CA. Reproductive and developmental effects of diethylene and triethylene glycol (methyl-, ethyl-) ethers. *Occupational hygiene*, 2:131–151(1996)

201-15023

Diglyme

CAS Number 111-96-6

$$Me\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}Me$$

ROBUST SUMMARIES

Existing Chemical	:	ID: 111-96-6
Memo	:	Ferro
CAS No.	:	111-96-6
EINECS Name	:	bis(2-methoxyethyl) ether
EC No.	:	203-924-4
TSCA Name	:	Ethane, 1,1'-oxybis[2-methoxy-
Molecular Formula	:	C6H14O3

Producer related part

Company	:	Ferro Incorporated, Grant Chem. Div.
Creation date	:	21.12.2003

Substance related part

Company	:	Ferro Incorporated, Grant Chem. Div.
Creation date	:	21.12.2003
Status	:	
Memo	:	
Printing date	:	29.12.2003
Revision date	:	
Date of last update	:	28.12.2003
Number of pages	:	36
Chapter (profile)	:	Chapter: 1.0.1, 1.2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1, 3.3.2, 3.5, 4.1, 4.2, 4.3, 4.4, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.7, 5.8.1, 5.8.2
Reliability (profile)	:	
Flags (profile)	:	

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	Toxicology and Regulatory Affairs
Contact person	:	Elmer Rauckman
Date	:	
Street	:	1201 Anise Court
Town	:	62243 Freeburg, IL
Country	:	United States
Phone	:	618-539-5380
Telefax	:	618-539-5394
Telex	:	
Cedex	:	
Email	:	rauckman@toxicsolutions.com
Homepage	:	ToxicSolutions.com

21.12.2003

1.2 SYNONYMS AND TRADENAMES

(2-METHOXYETHYL) ETHER

21.12.2003

2-(2-METHOXYETHOXY)-1-METHOXYETHANE

21.12.2003

BIS(2-METHOXYETHYL) ETHER

21.12.2003

DIETHYLENE GLYCOL DIMETHYL ETHER

21.12.2003

DIGLYCOL METHYL ETHER

21.12.2003

DIGLYME

21.12.2003

ETHANE, 1,1'-OXYBIS(2-METHOXY-

21.12.2003

ETHANOL, 2,2'-OXYBIS-, DIMETHYL ETHER

21.12.2003

ETHER, BIS(2-METHOXYETHYL)

21.12.2003

POLY-SOLV

21.12.2003

2.1 MELTING POINT

Value : = -68 °C

Test substance : Diglyme, CASNO 111-96-6
Reliability : (2) valid with restrictions

Handbook values are assigned a score of 2
Flag : Critical study for SIDS endpoint
21.12.2003 (16)

2.2 BOILING POINT

Value : = 162 °C at 1010 hPa

Test substance :
Diglyme, CASNO 111-96-6

Reliability : (2) valid with restrictions

Handbook values are assigned a score of 2
Flag : Critical study for SIDS endpoint
22.12.2003 (16)

2.3 DENSITY

Type :
Value : = .9451 g/cm³ at 20 °C

Test substance :
Diglyme, CASNO 111-96-6

Reliability : (2) valid with restrictions

Handbook values are assigned a score of 2
22.12.2003 (16)

2.4 VAPOUR PRESSURE

Value : = 3.49 hPa at 25 °C

Test substance :
Diglyme, CASNO 111-96-6

Reliability : (2) valid with restrictions

Handbook values are assigned a score of 2
Flag : Critical study for SIDS endpoint
22.12.2003 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= -.36 at 25 °C
pH value	:	
Test substance	:	Diglyme, CASNO 111-96-6
Reliability	:	(2) valid with restrictions Handbook values are assigned a score of 2
Flag	:	Critical study for SIDS endpoint

22.12.2003 (6)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	> 1000 g/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	not soluble
Stable	:	
Test substance	:	Diglyme, CASNO 111-96-6
Reliability	:	(2) valid with restrictions Handbook values are assigned a score of 2
Flag	:	Critical study for SIDS endpoint

22.12.2003 (16)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	= .0000000000175 cm³/(molecule*sec)
Degradation	:	= 50 % after 7.3 hour(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	
GLP	:	no
Test substance	:	

Method :

The structure was initially examined to determine if there was a chromophore that could absorb light energy at wavelengths above 295 um. As there is not, it was assumed that direct photolysis would be unimportant to the fate of the test material.

The indirect photolysis rate was then calculated from an experimental rate constant for diglyme found in the literature of 17.5 10-12 cm3/molecule-sec, assuming the equilibrium concentration of tropospheric hydroxyl radicals as 1,500,000 molecules of hydroxy radical per cm3.

The APOWIN program was also run to determine an estimated rate of reaction with hydroxyl radical. This was compared against the measured value as supporting data.

SMILES : COCCOCCOC
CHEM : Diglyme
MOL FOR: C6 H14 O3
MOL WT : 134.18

```
-------- SUMMARY (AOP v1.90): HYDROXYL RADICALS -------------
Hydrogen Abstraction       =  29.6904 E-12 cm3/molecule-sec
Reaction with N, S and -OH =   0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant =  29.6904 E-12 cm3/molecule-sec
   HALF-LIFE =     0.360 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     4.323 Hrs

Experimental Database Structure Match:
  Chem Name :  2-Methoxyethyl ether
  CAS Number:  000111-96-6
  Exper OH rate constant   :  17.5  E-12  cm3/molecule-sec
    Exper OH Reference:  ATKINSON,R (1989)
  Exper Ozone rate constant: --- cm3/molecule-sec
 Exper NO3 rate constant : --- cm3/molecule-sec
```

Hydrogen Abstraction Calculation:
Kprim = 0.136 F(-O-)=0.136(6.100)= 0.830
Ksec = 0.934 F(-CH2-)F(-O-)=0.934(1.230)(6.100)= 7.008
Ksec = 0.934 F(-O-)F(-CH2-)=0.934(6.100)(1.230)= 7.008
Ksec = 0.934 F(-CH2-)F(-O-)=0.934(1.230)(6.100)= 7.008
Ksec = 0.934 F(-O-)F(-CH2-)=0.934(6.100)(1.230)= 7.008
Kprim = 0.136 F(-O-)=0.136(6.100)= 0.830
H Abstraction TOTAL = 29.690 E-12 cm3/molecule-sec

Result :

The calculated half-life is 7.33 hours based on 1,500,000 molecules of hydroxyl radical per cc. The APOWIN program estimates a faster reaction rate with a subsequent estimated half-life of 4.3 hours. As these values are similar the experimental value is accepted for use.

SMILES : COCCOCCOC
CHEM : Diglyme
MOL FOR: C6 H14 O3
MOL WT : 134.18

```
------- SUMMARY (AOP v1.90): HYDROXYL RADICALS -------------
Hydrogen Abstraction       =  29.6904 E-12 cm3/molecule-sec
Reaction with N, S and -OH =   0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant =  29.6904 E-12 cm3/molecule-sec
   HALF-LIFE =     0.360 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     4.323 Hrs

-------- SUMMARY (AOP v1.90): OZONE REACTION ----------------

      ******  NO OZONE REACTION ESTIMATION ******
      (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  2-Methoxyethyl ether
  CAS Number:  000111-96-6
  Exper OH rate constant   :  17.5  E-12  cm3/molecule-sec
    Exper OH Reference:  ATKINSON,R (1989)
  Exper Ozone rate constant:  ---  cm3/molecule-sec
  Exper NO3 rate constant  :  ---  cm3/molecule-sec
```

Hydrogen Abstraction Calculation:
Kprim = 0.136 F(-O-)=0.136(6.100)= 0.830
Ksec = 0.934 F(-CH2-)F(-O-)=0.934(1.230)(6.100)= 7.008
Ksec = 0.934 F(-O-)F(-CH2-)=0.934(6.100)(1.230)= 7.008
Ksec = 0.934 F(-CH2-)F(-O-)=0.934(1.230)(6.100)= 7.008
Ksec = 0.934 F(-O-)F(-CH2-)=0.934(6.100)(1.230)= 7.008
Kprim = 0.136 F(-O-)=0.136(6.100)= 0.830
H Abstraction TOTAL = 29.690 E-12 cm3/molecule-sec

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

7.33 hours is accepted as the atmospheric half-life of diglyme in the troposphere due to indirect photolysis. No direct photolysis or reaction with atmospheric ozone is anticipated.

Reliability : (2) valid with restrictions

EPIWIN calculated values that are scientifically sound are assigned a reliability score of 2

Flag : Critical study for SIDS endpoint

22.12.2003 (1)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C
Deg. product :
Method : other: calculated and vehicle stability
Year :
GLP :
Test substance :

Method :

The water stability of this material may be reliably estimated from chemical principles. Aliphatic ethers linkages are one of the groups not considered susceptible to aqueous hydrolysis at environmental pH levels (Harris 1990).

Additional support for hydrolytic stability comes from an experimental approach that was used to demonstrate test material dosing vehicle stability by the NTP in aqueous solution.

Result :

The knowledge that ethers are not susceptible to hydrolysis allows prediction of a half-life > 1 year for diglyme.

Vehicle stability testing of diglyme prior to a developmental toxicity study indicated that diglyme was hydrolytically stable. Gas chromatography analysis of an aqueous solution at 47.2 g/L kept in the dark for 21 days, allowed the National Toxicology Program to conclude that diglyme is hydrolytically stable.

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

The hydrolysis half-life of diglyme at ambient temperatures and typical environmental pH levels is greater than one year.

Reliability : (2) valid with restrictions

A reliability code of 2 is assigned to values obtained from reliable estimation methods.

Flag : Critical study for SIDS endpoint

22.12.2003 (7) (18)

3.3.2 DISTRIBUTION

Media : other: air - water- soil -sediment
Method : Calculation according Mackay, Level III
Year :

Method :

Measured values for physical values of diglyme were input into EPIWIN as shown below. Biodegradation rates were estimated from limited experimental results. Model was allowed to assume equal distributions to air, water and soil. EQC Level III model (as found in EPIWIN 3.05) was utilized.

Result :

```
Results of the Level III fugacity modeling are:

Level III Fugacity Model (Full-Output):
=======================================
Chem Name   : Diglyme
Molecular Wt: 134.18
Henry's LC  : 5.23e-007 atm-m3/mole (calc VP/Wsol)
Vapor Press : 2.96 mm Hg  (user-entered)
Log Kow     : -0.36  (user-entered)
Soil Koc    : 0.179  (calc by model)

       Concentration  Half-Life    Emissions
          (percent)      (hr)       (kg/hr)
 Air       0.505         7.33         1000
 Water     60.4          2e+003       1000
 Soil      38.9          2e+003       1000
 Sediment  0.113         4e+003       0

       Fugacity   Reaction  Advection Reaction  Advection
        (atm)     (kg/hr)   (kg/hr)   percent) (percent)
Air    1.87e-011   970       103       32.3      3.42
Water  2.39e-011   425       1230      14.2      40.9
Soil   5.62e-010   274       0         9.13      0
Sed    2.22e-011   0.396     0.0458    0.0132    0.00153

   Persistence Time: 677 hr
   Reaction Time:    1.22e+003 hr
   Advection Time:   1.53e+003 hr
   Percent Reacted:  55.7
   Percent Advected: 44.3

   Half-Lives (hr), (based upon user-entry):
      Air:      7.33
      Water:    2000
      Soil:     2000
      Sediment: 4000

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

Under conditions of equal initial distribution to water, soil and air, Biphenyl is expected to distribute preferentially in water > soil > air > sediment.

Reliability : (2) valid with restrictions

A reliability code of 2 is assigned to values obtained from reliable estimation methods.

Flag : Critical study for SIDS endpoint

23.12.2003 (2)

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, non-adapted
Concentration	:	2 mg/l related to Test substance related to
Contact time	:	
Degradation	:	(±) % after
Result	:	under test conditions no biodegradation observed
Deg. product	:	
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	
GLP	:	
Test substance	:	

Method :

An OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test" was conducted on diglyme at a concentration of 2 mg/L using an activated sludge from an unspecified source. The degradation was reported as 0.1% after 5 days but no 28-day result was provided.

Remark :

Result supported by the following studies indicating diglyme is difficult to biodegrade:

Tessier, S et al. Degradation of Polyoxyethylenes: Biodegradation using an Enzyme from Psuedomonas P-400 and Chemical Degradation using Sodium Hypochlorite. J. Chem research 1983: 174-175 (1983)

Roy, D. Anagnostu, G. Chaphalkar, P. Biodegradation of Dioxane and Diglyme in Industrial Waste. Journal of Environmental Science and Health Part A Environmental Science And Engineering; 29 (1). 1994. 129-147

Harada T and Y Nagishima, Utilization of Alkylether Compounds by Soil Bacteria. J. Fement. Tech. 53:218-222 (1975)

Cowan RM, et al. Activated Sludge and Other Aerobic Suspended Culture Processes Water Environment Research; 67 (4). 1995. 433-450

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

Not readily biodegradable

Reliability : (2) valid with restrictions

Guideline study, but original report was not available for review. Study was also indicated as not GLP.

Flag : Critical study for SIDS endpoint

24.12.2003 (13)

Type : aerobic
Inoculum : activated sludge, industrial, non-adapted
Concentration : 375 mg/l related to DOC (Dissolved Organic Carbon)
related to
Contact time : 28 day(s)
Degradation : = 31 - 42 (±) % after 28 day(s)
Result :
Deg. product :
Method : OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year : 1989
GLP :
Test substance :

Method :

An OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test" was conducted on diglyme. The initial concentration of diglyme, based on dissolved organic carbon, was 375 mg/L. The bacterial culture was non-adapted industrial activated sludge.

Result :

Results are given as follows in the IUCLID-2000 document:

TIME	Degradation (%)
3 hours	0
1 day	10
7 days	10
15 days	15
20 days	36
28 days	31

Results are cited in the IPCS CICAD document:

"...adsorption of diglyme onto activated sludge was 17% after 3 h, and total removal was 42% after 28 days. The degree of elimination and the degradation curve are indicative of inherent primary degradation, according to OECD criteria (Hoechst, 1989a)."

Both results are attributed to Hoechst AG; however, it appears the CICAD may reference a secondary Hoechst document.

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

Not inherently biodegradable but the degree of elimination and the degradation curve are indicative of inherent primary degradation

Reliability : (2) valid with restrictions

Guideline study, but original report was not available for review. Study was also indicated as not GLP.

Flag : Critical study for SIDS endpoint

24.12.2003 (9) (13)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 2000 measured/nominal
LC0	:	> 2000 measured/nominal
Method	:	other: DIN 38412, L15 guideline
Year	:	
GLP	:	no
Test substance	:	

Method :

An acute fish toxicity test was performed according to the DIN 38412, L15 guideline.

Remark :

It could not be determined how the concentration of the test substance was established in this study, or if only nominal concentrations were used. The use of measured concentrations is not considered to be of much importance for this water soluble, low volatility, and low adsorption potential test material. It can be assumed that all nominal concentrations of the test substance are expected to correspond closely to actual concentrations, even using open systems and longer exposure periods.

This study is supported by the EPIWIN predicted 96-hour LC50 for freshwater fish of 16,650 mg/L

Result :

No mortality was observed in the 96-hour study. No adverse effects were seen in the in-life phase of this study. Fish were sectioned at the end of the study and no visible changes were apparent.

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

The acute LC0 and LC50 for the golden orfe is > 2000 mg/L under these conditions

Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

28.12.2003 (12)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 1000 measured/nominal
EC0	:	> 1000 measured/nominal
Limit Test	:	no
Method	:	OECD Guide-line 202
Year	:	
GLP	:	no data

	:	
Method	:	An acute toxicity test with D. magna was performed according to the OECD 202 guideline.
Remark	:	It could not be determined how the concentration of the test substance was established in this study, or if only nominal concentrations were used. The use of measured concentrations is not considered to be of much importance for this water soluble, low volatility, and low adsorption potential test material. It can be assumed that all nominal concentrations of the test substance are expected to correspond closely to actual concentrations, even using open systems and longer exposure periods. This study is supported by the EPIWIN predicted 48-hour EC50 for Daphnia magna of 14,971 mg/L
Result	:	No adverse effects were observed at concentrations of 100 or 1000 mg/L.
Test substance	:	Diglyme, CASNO 111-96-6
Reliability	:	(2) valid with restrictions Guideline study, but original report was not available for review. GLP status could not be determined.
Flag	:	Critical study for SIDS endpoint

28.12.2003 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
EC10	:	> 1000 measured/nominal
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	
GLP	:	
Remark	:	It could not be determined how the concentration of the test substance was established in this study, or if only nominal concentrations were used. The use of measured concentrations is not considered to be of much importance for this water soluble, low volatility, and low adsorption potential test material. It can be assumed that all nominal concentrations of the test substance are expected to correspond closely to actual concentrations, even using open systems and longer exposure periods. This study is supported by the EPIWIN predicted 96-hour EC50 for green algae of 8171 mg/L
Test substance	:	Diglyme, CASNO 111-96-6
Reliability	:	(2) valid with restrictions Guideline study, but original report was not available for review. GLP status could not be determined.
Flag	:	Critical study for SIDS endpoint

28.12.2003 (11)

5.1.1 ACUTE ORAL TOXICITY

Type	:	other: Approximate Lethal Dose
Value	:	ca. 7500 mg/kg bw
Species	:	rat
Strain	:	CD-1
Sex	:	male
Number of animals	:	8
Vehicle	:	water
Doses	:	1500 to 23000 mg/kg
Method	:	
Year	:	
GLP	:	no data

Method :

Young adult ChR-CD male rats were administered the test material by intragastric intubation as a water solution in single doses. Surviving animals were observed for 14 days and were sacrificed and necropsied.

Remark :

This result is supported by a GLP study reported in IUCLID-2000 in which female Wistar rats were treated with doses of 1600, 2500, 4000, 4500, 5600 or 6300 mg/kg diglyme. Clinical signs were restlessness unrest, disturbed sense of balance, prone position and reduced respiration rate. At the higher dose levels there was also discharge of a reddish secretion from the eye sockets. These signs only persisted for 24 hours in animals that survived the 14-day observation period.

At > = 4500 mg/kg deaths occurred 24 to 96 hours afer dosing.
At < = 4000 mg/kg there were no deaths.

Body weight development of survivors was normal after 14 days. The lungs of animals dying on test showed a gray-red colouring and were partly dark red spotted; some animals showed liver changes. No findings were reported from the 14-day necropsy of surviving animals.

The Oral LD50 was determined to be 4760 mg/kg

Hoechst (1979b) Akute orale Toxizität von Diethylenglykoldimethylether an weiblichen Ratten. Frankfurt am Main, Hoechst AG, 4 pp. (Report 376/79; unpublished). Study details as reported in IUCLID-2000 Document in German.

Result : Results are given in the table

Dose	% in water	Mortality	Time of Death
23,000	80	D	1 hour
17,000	80	D	3 hours
11,000	80	D	1 day
7,500	80	D	2 days
5,000	50	S	14 days
3,400	50	S	14 days
2,250	50	S	14 days
1,500	50	S	14 days

D= Died
S= Survived to terminal sacrifice

CLINICAL EFFECTS:

@ Lethal Doses: Ataxia, loss of muscle tone, lethargy, prostration, labored respirations. and belly-to-cage posture at 7,500 mg/kg and above; pallor at 17,000 mg/kg and above; loss of righting reflex at 25,000 mg/kg.

@ Nonlethal Doses: Lethargy, belly-to-cage posture and ruffled fur at 1,500 mg/kg and above; prostration at 5,000 mg/kg on day of dosing. Ruffled fur at 5,000 mg/kg on day after dosing; weight loss 1-2 days at 2,250 mg/kg and above.

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

Diglyme is slightly toxic when administered orally to young adult ChR-CD male rats in single doses; its Approximate Lethal Dose (ALD) is 7,500 mg/kg of body weight.

Reliability : (2) valid with restrictions

Study was conducted by a scientifically defensible procedure and results are supported by other data.

Flag : Critical study for SIDS endpoint

28.12.2003 (5)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : > 11000 mg/m³
Species : rat
Strain : Wistar
Sex : male/female
Number of animals :
Vehicle :
Doses :
Exposure time : 7 hour(s)

Method :

Wistar rats of each sex were exposed to diglyme vapors in an "inhalation risk test" using air "saturated" with diglyme and an exposure period of 7 hours. After exposure, animals were maintained for a 14-day observation period after which they were sacrificed and subjected to a gross necropsy.

Remark :

The CICAD document for diglyme states that this was a nose-only exposure but the IUCLID-2000 document does not provide details about the method of exposure. In the IUCLID-2000 document details are given about the nominal exposure concentration in which 46.8 grams of test material were used in the 7 hour exposure with a flow rate of 600 L/hr. This calculates to 11.14 grams per cubic meter or about 2000 ppm. As the vapor pressure of diglyme is 2.96 mm Hg at 25 C, saturated air could contain up to 3900 ppm diglyme. Given the difficulty in saturating air with test material using a flow-though apparatus, the 2000 ppm nominal concentration is reasonable.

This result is supported by the results of repeated-dose studies reported by McGregor et al. (1983)*, who exposed CD rats for 7 hours a day for 5 days

at a measured diglyme concentration of 1000 ppm without mortality. It is also supported by the work of Valentine et al. (1999) who exposed groups of 20 male and 10 female rats to measured concentrations of up to 1100 ppm diglyme, six hours a day five days a week for 10 exposures without mortality.

*McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW (1983) Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. Toxicology and Applied Pharmacology, 70:303-316.

Valentine R, O'Neill AJ, Lee KP, Kennedy GL Jr. (1999) Subchronic inhalation toxicity of diglyme. Food Chem Toxicol. 37:75-86.

Result :

All animals survived exposure and a 14-day observation period. No macroscopic findings were observed at necropsy 14 days after the exposure. Clinical signs were restlessness, narrowing of palpebral fissures, and irregular breathing in rats. Based on the vapor pressure the concentration could have been as high as 3800 ppm (21 mg/L) but in the IUCLID 2000 summary is given as > 11 grams/m3. This is based on the nominal concentration calculated by the weight loss of test material and the flow rate of the apparatus. The actual nominal concentration calculates to 11.14 mg/L or about 2000 ppm

Test substance :

Diglyme, CASNO 111-96-6

Reliability : (2) valid with restrictions

Study was conducted by a scientifically defensible procedure and results are supported by other data.

Flag : Critical study for SIDS endpoint

28.12.2003 (8) (14)

5.1.3 ACUTE DERMAL TOXICITY

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	other: Crl:CD1BR
Route of admin.	:	inhalation
Exposure period	:	10 exposures in 2 weeks
Frequency of treatm.	:	5 days per week
Post exposure period	:	Up to 84 days for males and 14 days for females
Doses	:	110, 370 or 1100 ppm
Control group	:	other: concurrent negative (air) and positive 300 ppm (2-methoxyethanol)
LOAEL (males)	:	= 110 ppm
Method	:	
Year	:	
GLP	:	yes

Method :

Crl:CD1BR rats (7 weeks old), received from Charles River Breeding Laboratories (Kingston, NY, USA), were maintained on a 12-hr/12-hr light/dark cycle at a targeted temperature of 21-25 deg C 28C and relative humidity of 40-60%. Except during exposure, Purina 5002 rat feed and water were available ad lib.

Five groups of 20 male and 10 female rats were used in this study. Three groups were exposed nose-only to target concentrations of 100, 300 or 1000 ppm diglyme. 2-Methoxyethanol at a target concentration of 300 ppp was used as a positive control on a group of the same size. The control group was exposed to air only. Rats were randomly assigned to treatment groups using a computer-based randomization and body weights. During exposure, rats were individually restrained in perforated, stainless-steel cylinders with conical nose pieces. Restrainers were inserted into face-plates on 150-litre exposure chambers such that the nose of each rat protruded into the chamber. Exposures were 6 hours a day and 5 days a week for 2 weeks. A recovery period lasting up to 84 days was utilized for some animals. Five rats of each sex were sacrificed after the 10th exposure and after 2 weeks recovery. Five male rats per group were also sacrificed after 6 and 12 weeks of recovery.

Test material or positive control substance vapors were generated by pumping the liquid test material through Teflon tubing using Harvard Model 975 compact infusion pumps into three-neck, glass, round-bottomed mixing flasks. For diglyme the flask was heated to 111-123 C and for 2-methoxyethanol the flask was heated to 79-87 C to facilitate evaporation.. Conditioned and filtered air was added to the mixing flask at approximately 35-46 liters/min to dilute and sweep the vapor through unheated glass connecting tubes into the inlets of the 150-liter exposure chambers. Exposure chamber concentrations were adjusted by varying the test material feed rate into the mixing flasks.

Chamber concentrations were determined at approximately 30-min intervals during each exposure. For diglyme analysis, known volumes of the chamber atmospheres were drawn from the rats' breathing zone through tandem glass impingers containing acetone as the trapping solvent. For 2-methoxyethanol analyses, replicate gas samples (approx. 0.5 ml) were collected from the breathing zone of the rats with a gas-tight syringe. Samples were analyzed by gas chromatography using a fame ionization detector. Exposure concentrations were calculated with standard curves prepared daily. Chamber temperature and relative humidity were measured regularly during each exposure.

Experimental observations.
All rats were weighed and observed for clinical signs daily and for the 14-day post-exposure recovery period. Male rats assigned to the extended recovery groups were also weighed and observed at least once a week for up to 12 weeks after the last exposure.

Urine samples were collected only from rats that were assigned to be sacrificed from those within 24 hours of urine collection. Urine specimens were collected overnight from five rats of each sex per exposure group after the 9th exposure and on the 13th day of recovery. Urine was also collected form the long-term male recovery rats on the 41st day of recovery and on the 83rd day of recovery. Samples were analyzed for volume,

osmolality, urobilinogen, pH, haemoglobin or
occult blood, glucose, protein, bilirubin and ketone.

Blood samples (from rats lightly anaesthetized with carbon dioxide) were taken from the orbital sinus of five rats of each sex per group after the 10th exposure and on the 14th day of recovery, and from five male rats per group on the 42nd day of recovery, and on the 84th day of recovery. Blood samples were analyzed for erythrocyte count, haemoglobin concentration, haematocrit, platelet count, leucocyte count, and relative numbers of neutrophils, band neutrophils, lymphocytes, atypical lymphocytes, eosinophils, monocytes and basophils. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were calculated from the erythrocyte data. Serum activities/levels of the following were determined :

alkaline phosphatase (AP),
alanine aminotransferase (ALT),
aspartate aminotransferase (AST),
urea nitrogen,
creatinine,
total protein
cholesterol

Rats were sacrificed as assigned by sodium pentobarbital anesthesia and exsanguination for gross and histopathological examinations. The lungs, liver, kidneys, spleen and male reproductive organs (testes, epididymides, seminal vesicles and prostate) were weighed at necropsy. Bone, eyes testes and epididymides were fixed in Bouin's solution. All other organs and tissue were fixed in 10% formalin solution. Paraffin sections were prepared according to standard laboratory SOPs. All sections were stained with hematoxylin and eosin. In addition, all testes were stained with the PAS method.

Representative samples of the following tissues were prepared for microscopic examination: heart, lungs, mesenteric and mediastinal lymph nodes, nasal cavities, trachea, liver, pancreas, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, kidneys, urinary bladder, bone/bone marrow (sternal), spleen, thymus, thyroid gland, adrenal glands, brain, eyes, testes, epididymides, prostate, seminal vesicles, vagina, ovaries, uterus, and any other organs or tissues with gross lesions.

Statistical analyses: Mean body weights, body weight gains, absolute organ weights, and relative organ weights (organ to body weight ratios) of treated animals were compared with control rats during the exposure and recovery periods. Data were statistically analyzed by one-way analysis of variance. Exposure group values were compared with controls by the least significant difference or Dunnett's tests when the ratio of variance (F) indicated a significant among-to-within group variation. Differences were considered significant at the 0.05 probability level. Clinical pathology data were analyzed by a one-way analysis of variance and Bartlett's test. When the F-test was significant, Dunnett's test was used to compare means from the control group with each treatment.

Result :

EXPOSURES

Analytical glyme concentrations were 110, 370 and 1100 ppm. The mean concentration of 2-methoxyethanol was 300 ppm.

In the diglyme exposure chambers temperatures ranged from 23 to 35 deg. C and relative humidity varied from 42 to 77%. In the 2-methoxyethanol chamber, temperature ranged from 22 to 32 deg C and relative humidity varied from 41 to 61%. In the air control chamber, temperature ranged from 22 to 28 deg C, relative humidity varied from 38 to 51%. The maximum observed temperature of 35 C was a transient excursion and occurred only once in the 300 ppm diglyme group. Restrainer temperatures were at ambient conditions.

CLINICAL OBSERVATIONS
At the high concentration, seven males had colored ocular discharge and 17 males and one female had diarrhea during the exposure phase. Diarrhea was observed in eight males from the 300 ppm 2-methoxyethanol group. Diarrhea and ocular discharge were transient clinical signs first noted during week 1 or 2 of exposure. No other chemical-related clinical observations were observed.

BODY WEIGHTS
Mean body weights of male rats from all test groups were lower than controls throughout the exposure period. Body weight gains were less with higher exposure levels. Weight losses in rats exposed to 2-methoxyethanolwere greater than in the 370 ppm diglyme group but lower than the 1100 ppm diglyme group. Weight recovery began after the10th exposure such that mean body weights were comparable to control values by recovery day 14 for the 110 ppm group rats, by recovery day 28 for the 370 ppm group, and by recovery day 42 for the 1100 ppm diglyme and 300 ppm 2-ME groups. No significant body weight differences were observed in female rats throughout the study.

BODY WEIGHTS, MALES

		-------DIGLYME----			2-ME
	0	110	370	1100	300ppm
Ex Day					
1	246	244	245	244	239
3	259	246*	245*	232*	240*
5	271	257*	257*	233*	243*
8	297	285*	284*	246*	277*
12	312	280*	285*	250*	270*
rd-7	362	342*	338*	291*	330*
rd-14	398	385	377*	342*	371*
rd-28	491	463	476	438	461
rd-42	533	524	577	530	531
rd-84	582	570	632	573	571

* p < 0.05

BODY WEIGHTS, FEMALES

		-------DIGLYME----			2-ME
	0	110	370	1100	300ppm
Ex Day					
1	187	192	191	189	185
3	190	193	192	180*	189
5	195	200	198	187	194
8	200	208	212	202	211
12	203	213	214	202	208
rd-7	238	244	236	228	246
rd-14	247	252	238	239	253

* p < 0.05

CLINICAL PATHOLOGY:
Male and female rats exposed to 1100 ppm diglyme or 300 ppm 2-ethoxyethanol were moderately anemic showing a reduction in red blood cell count, hemoglobin and hematocrit after the 10th exposure. After 14 days of recovery, clinical signs of anemia were not detected in any treatment group
.
Platelet counts of male and female rats from several of the diglyme and 2-methoxyethanol groups were reduced after 14 days of recovers but not on day-10 after the last exposure. Platelet counts returned to control values by 42 days of recovery in male rats.

Compared with control values, mean leukocyte counts were reduced in male rats from all test groups and in female rats from the 1100 ppm diglyme and 300 ppm 2-methoxyethanol groups after the last exposure. Lymphopenia was concentration related, with the most severe effects seen in the 1100 ppm diglyme group. Lymphopaenia was also observed in the 1100 ppm diglyme and 300 ppm 2-methoxyethanolfemales.

Serum activities of ALT, AST and AP and total protein concentrations were reduced in male and female rats exposed to 1100 ppm diglyme compared with controls after the 10th exposure. Similar, but less severe, effects were noted in male and female rats exposed to 2-methoxyethanol. After 14 days of recovery, serum hepatic enzyme activities and serum protein concentrations were similar to control values in all test groups.

ORGAN WEIGHTS:
Absolute and relative weights of male reproductive organs were reduced in diglyme and 2-methoxyethanol exposed rats compared with controls (see table for absolute weights). The extent of the effects was generally concentration related, with the most severe effects observed in the 1100 ppm diglyme and the 300 ppm 2-methoxyethanol groups. A concentration-related reduction in prostate and seminal vesicle weights occurred in the 370 and 1100 ppm diglyme and 300 ppm 2-methoxyethanol groups.

Testes weights were lower in the 1100 ppm diglyme and 300 ppm 2-methoxyethanol groups. After 14 days recovery, mean absolute testes and epididymides weights in the 370 and 1100 ppm diglyme and 300 ppm 2-methoxyethanol groups and prostate weights in the 1100 ppm diglyme and 300 ppm 2-methoxyethanol groups remained lower than controls. After 42 and 84 days of recovery, mean testes weights in the 1100 ppm diglyme and 300 ppm 2-methoxyethanol groups were lower than control values;

mean absolute testes weights were also lower than controls in the 110 and 370 ppm diglyme groups after 42 days of recovery and mean relative epididymides weights were lower than controls in the 370 ppm diglyme group after 84 days of recovery.

In female rats, the only significant weight changes associated with diglyme exposure was increased mean absolute and relative liver weights in the 1100 ppm group and increased mean absolute liver weights in the 370 ppm group after the 10th exposure.

ORGAN	Test day	0	------DIGLYME----- 110	370	1100	2-ME (300)ppm
Testes	10	2.88	2.75	2.64	1.45*	1.76*
	R14	3.10	2.89	2.66*	1.08*	1.43*
	R42	3.41	3.05	2.85*	1.33*	1.98*
	R84	3.49	3.29	3.40	2.69*	2.85*
Prostate	10	0.58	0.53	0.40*	0.33*	0.34*
	R14	0.79	0.82	0.87	0.64*	0.65*
	R42	1.17	1.28	1.23	1.09	1.02
	R84	1.49	1.36	1.61	1.40	1.56
Seminal vesicles	10	1.38	1.23	1.07*	0.92*	0.92*
	R14	1.83	1.96	1.40	1.52	1.65
	R42	2.75	2.00	2.53	2.61	2.74
	R84	3.28	2.76	2.86	2.76	2.91
Epididym.	10	0.77	0.86	0.80	0.70	0.68
	R14	1.06	1.06	0.93*	0.66*	0.79*
	R42	1.38	1.29	1.23	0.84*	1.04*
	R84	1.60	1.59	1.52	1.18*	1.36*

GROSS PATHOLOGY:
After 10 exposures, the thymus and reproductive organs (testes, prostate, seminal vesicles and epididymides) of male rats exposed to 1100 ppm diglyme or 300 ppm 2-methoxyethanol were smaller than controls. After 42 days of recovery, the prostate, seminal vesicles and thymus appeared normal at necropsy in both these groups; however, testes and epididymides in rats from these groups were observed to be small at all examination times. Overall, the gross lesions in male rats were consistent with the microscopic observations. These observations were noted in rats exposed to either 100 or 300 ppm diglyme.

No compound-related gross lesions were noted upon necropsy in female rats.

MICROSCOPIC EXAMINATION

DIGLYME
After 10 diglyme exposures at 110 ppm, slight testicular atrophy was observed microscopically in two of five male rats, while two of five controls had minimal testicular atrophy. In both groups, the epididymal tubules contained exfoliated degenerative germ cells. No control rats had testicular atrophy following 14, 42 or 84 days recovery. After 14 days recovery, two of five rats exposed to 110 ppm diglyme were observed to have slight testicular atrophy. After 42 days recovery, two of five rats in this group showed minimal testicular injury involving a small number of atrophic tubules; most seminiferous tubules had normal germinal epithelium. After 84 days recovery, the testicular germinal epithelium morphology was normal.

After 10 diglyme exposures at 370 ppm, slight testicular atrophy primarily affecting spermatocytes and immature spermatids were seen in all males. After 14 days of recovery, minimal to moderate testicular atrophy was present in all five rats. Immature spermatids and spermatocytes were found, but mature spermatozoa had not yet developed. Exfoliated degenerative germ cells and a slightly reduced number of spermatozoa were found in the epididymal tubules. After 42 days recovery, only the testes of two of five rats exhibited very slight testicular atrophy. After 84 days recovery, the morphology of the testicular germinal epithelial was since considered normal.

After 10 diglyme exposures at 1100 ppm all males showed severe testicular atrophy. The germinal epithelium showed extensive damage and all spermatogenic stages of germ cells were affected; numerous spermatid giant cells were also present. The epididymal tubules showed numerous exfoliated degenerative germinal cells and slight to moderate oligospermia. After 14 days recovery, the seminiferous tubules showed slight regeneration of spermatocytes and spermatids. Some seminiferous tubules were lined with only Sertoli cells and a few spermatogonia. Slight Leydig cell hyperplasia was present in all rats. The epididymal tubules contained numerous spermatid giant cells with only a few spermatozoa (moderate to severe oligospermia). After 42 days recovery, many tubules showed a regenerating germinal epithelium consisting of spermatocytes and immature spermatids although minimal to moderate Leydig cells hyperplasia persisted. The epididymal tubules contained numerous exfoliated germinal cells and few spermatozoa (moderate to severe oligospermia). After 84 days recovery, three of four rats had almost normal germinal epithelium, but the remaining rat showed moderate testicular atrophy with only partially regenerated germinal epithelium. In addition to the testicular effects, the seminal vesicles and prostate were atrophic after 2 wk of exposure but these effects had reversed by 14 and 42 days of recovery, respectively. Minimal to severe bone marrow hypoplasia and lymphoid tissue atrophy of the spleen and thymus were apparent in both male and female rats exposed to 1100 ppm diglyme. Atrophic changes in the hematopoietic tissues of rats from this group resolved after 14 days of recovery; however, extramedullary hematopoietic foci were evident in the liver of rats of each sex and in the spleens of males. Evidence of hematopoietic effects in males was essentially absent after 42 days of recovery.

2-METHOXYETHANOL
2-Methoxyethanol exposure also produced adverse effects in hematopoietic tissues of male and female rats and testicular injury in males. Ten exposures to 300 ppm 2-methoxyethanol for produced a transient, minimal to moderate atrophy of thymic lymphoid tissues in males and females and atrophy of splenic lymphoid tissues in males; these effects were not evident after 14 days recovery. Slight to severe testicular atrophy was a finding in all rats killed at the end of the exposure period. The germinal cells (spermatocytes) were the primary cell type damaged. The magnitude of the testicular injury produced by 300 ppm 2-methoxyethanol was more severe than that in the 370 ppm diglyme group but slightly less severe than that produced by 1100 ppm diglyme. After 14 days recovery, male rats had severe testicular atrophy, although slight regeneration of germinal epithelium, spermatocytes and immature

spermatids was noted in seminiferous tubules. In addition, minimal to mild Leydig cell hyperplasia was observed in all five rats. The epididymal tubules contained exfoliated degenerative germinal cells, spermatid giant cells, and a decreased number of spermatozoa (severe oligospermia). After 42 days recovery, many tubules had normal germinal epithelium, but slight atrophy persisted. Some tubules exhibited regenerating spermatocytes or immature spermatids, but were devoid of mature spermatids and spermatozoa. Severely damaged tubules were lined with Sertoli cells and a few spermatogonia or spermatocytes. Epididymal tubules were filled with numerous exfoliated germinal cells and a few spermatogonia (moderate to severe oligospermia). After 84 days of recovery, most of the testes had normal germinal epithelium morphology, but some testes still had a few seminiferous tubules showing incompletely regenerated germinal epithelium. The prostate and seminal vesicles exhibited a slightly atrophic structure after 10 exposures and 14 days of recovery, but had regained the normal structure by 42 days recovery.

Test substance :

Diglyme, CASNO 111-96-6 >99% pure

Conclusion :

Administration of diglyme by inhalation produced a variety of concentration-related effects to the reproductive system of male rats and the hematopoietic system of male and female rats. Adverse effects occurred at lower exposure concentrations in males than in females.

The reproductive and hematopoietic effects were generally reversible, although complete recovery from testicular injury was not observed in some rats exposed to the highest concentration of diglyme. The NOEL for repeated exposure to diglyme in female rats is 370 ppm, while a NOEL for male rats was not demonstrated (<110 ppm).

Diglyme appeared to be approximately two- to threefold less potent than 2-methoxyethanol on a molar basis.

Reliability : (1) valid without restriction

Modern guideline-like GLP study with full documentation available.

Flag : Critical study for SIDS endpoint

26.12.2003 (19)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Salmonella typhimurium reverse mutation assay
System of testing	:	
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: NTP Protocol
Year	:	
GLP	:	yes
Test substance	:	

Method :

As each stain of Salmonella typhimurium is genetically different, using several strains in a test increases the opportunity of detecting a mutagenic chemical. All strains of Salmonella typhimurium used for mutagenicity

testing carry a defective (mutant) gene that prevents them from synthesizing the essential amino acid histidine. Mutations leading to the ability to sysntesize histidine are called "back" or "reverse" mutations and the process is referred to as "reversion."
Some test protocols utilize extracts of Aroclor rat or hamster liver enzymes (S9) to promote metabolic conversion of the test chemical. This is necessary since the Salmonella bacterium does not have the mamillian metabolic capabilities.
In the Salmonella assay, a test tube containing a suspension of one strain of Salmonella typhimurium plus S9 mix or plain buffer without S9, is incubated for 20 minutes at 37º C with the test chemical. Control cultures, with all the same ingredients except the test chemical, are also identically incubated. In addition, positive controls with a known potent mutagen, are prepared. After 20 minutes, agar is added to the cultures and the contents of the tubes are thoroughly mixed and poured onto the surface of petri dishes containing standard bacterial culture medium. The plates are incubated, and bacterial colonies that do not require an excess of supplemental histidine appear and grow. These colonies are comprised of Salmonella that have undergone reverse mutation to restore function of the histidine-manufacturing gene. The number of colonies is counted after 2 days.

Several doses (at least 5) of each test chemical and multiple strains of Salmonella typhimurium are used in each experiment. In addition, cultures are set up with and without added S9 liver enzymes at 10% concentration I these studies.
The pattern and the strength of the mutant response are taken into account in determining the mutagenicity of a chemical. All observed responses are verified in repeat tests. If no increase in mutant colonies is seen after testing several strains under several different culture conditions, the test chemical is considered to be nonmutagenic in the Salmonella test.

Reference
Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000 Nov 20;455(1-2):29-60.

Remark :

This result is supported by negative results from three other Ames tests. McGregor reported two and the other is contained in two unpublished reports form Hoechst AG

References for supporting studies:
McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW (1983) Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. Toxicology and applied pharmacology, 70:303-316 .

Hoechst (1979d) Test for mutagenicity in bacteria strains in the absence and presence of a liver preparation. Frankfurt am Main, Hoechst AG, 7 pp. (Report 53/79; unpublished). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

Hoechst (1979e) Mutagenicity evaluation of diethyleneglycoldimethylether in the Ames Salmonella/microsome plate test. Frankfurt am Main, Hoechst AG, 15 pp. (Report 743/79, unpublished). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

Result :

Summary Information for Diglyme (111-96-6)
Study Vehicle: Water
Protocol: Preincubation
Result: Negative

Strain: TA100

Dose	No MA (equiv)		No MA (neg)		10% RLI (neg)		10% RLI (neg)		10% HLI equiv)		10% HLI (neg)	
ug/Pl	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	142	10	104	19.4	199	5	139	7.7	191	10.1	141	6.2
100	138	10.1	105	3.5	184	1.5	121	3.9	188	9.5	133	4.9
333	164	6.2	96	12.9	196	22.3	124	6.7	185	25.8	136	2.4
1000	189	11.5	103	17.9	237	18.2	151	14.2	228	8.1	148	13.5
3333	179	14.6	107	12.2	219	3.2	142	5.5	254	7.8	150	18.9
10000	180	9.9	85	1.7	222	24.3	159	12	251	18.9	160	12.4
Pos Co	390	42.2	287	6.8	498	10.3	326	12.2	650	90.7	547	20.3

Strain: TA1535

Dose	No MA (neg)		No MA (neg)		10% RLI (neg)		10% RLI (neg)		10% HLI (neg)		10% HLI (neg)	
ug/Pl	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	10	1.2	9	1.2	15	0.7	11	2.6	11	1.5	8	0.6
100	8	1.2	7	1.2	8	2.7	7	2.3	11	2.1	8	1.5
333	10	1.5	9	1.5	8	1.7	6	1.2	8	1.2	5	1
1000	8	2.2	8	1.9	13	1.5	10	1.2	8	2.2	6	1.5
3333	9	1.5	8	1.5	9	2.1	7	1.5	10	0.9	6	1.5
10000	7	0.9	6	1	7	2.1	5	1.5	7	1.7	5	1.2
Pos Co	229	8	232	12.5	56	10.7	29	3.2	62	8.2	87	7.4

Strain: TA1537

Dose	No MA (neg)		No MA (neg)		10% RLI (neg)		10% RLI (neg)		10% HLI (neg)		10% HLI (neg)	
ug/Pl	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	8	1.5	6	1.2	7	2.3	7	1.7	9	1.5	9	0.3
100	5	2.6	5	1.3	8	1.9	8	1.5	9	1.3	7	2.3
333	6	0	5	1.2	7	2	7	1.2	8	1.2	6	1.2
1000	7	1.2	6	0.6	8	2.3	9	0.3	7	0.9	7	0.3
3333	5	0.6	5	2.3	8	1.8	8	0.9	4	0.3	7	1.8
10000	6	1.2	6	0.7	12	0.6	8	2.1	7	2.2	8	0.7
Pos Co	496	32	218	19.9	88	14	31	3.5	134	6.4	79	8.4

Strain: TA98

Dose	No MA (neg)		No MA (neg)		10% RLI (neg)		10% RLI (neg)		10% HLI (neg)		10% HLI (neg)	
ug/Pl	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	13	0.9	14	0.7	24	3.8	20	1.2	28	3	19	1.5
100	14	2.1	15	1.5	21	1.5	19	1.2	27	1.8	21	2
333	14	1.5	15	1.7	21	1.9	16	1.8	27	0.7	21	1.2
1000	15	1	16	0.3	20	4	18	1.2	21	2.6	17	1.2
3333	16	2.6	17	0.3	30	2.9	21	3.1	21	2	17	1
10000	23	2.6	18	4.1	29	0.3	19	2.6	24	2.3	22	2.7
Pos Co	374	18.7	209	27	529	48.8	125	7.6	905	73.9	251	10.7

S = Slight Toxicity
MA = Metabolic Activation
RLI = Rat Liver, Induced
HLI = Hamster Liver, Induced

Test substance :

Diglyme, CASNO 111-96-6

Conclusion :

Material was non-mutagenic in the presence or absence of a standard liver metabolic activating system

Reliability : (1) valid without restriction
NTP guideline study using optimized conditions

Flag : Critical study for SIDS endpoint

22.12.2003 (3)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	7 hr/day for 1 or 5 days
Doses	:	0, 250 or 1000 ppm
Result	:	negative
Method	:	
Year	:	
GLP	:	
Test substance	:	

Method :

CD rats (Sprague-Dawley derived) were used and were obtained from Charles River (UK)

Test atmospheres were generated by bubbling dry oxygen-free nitrogen through diglyme contained in a Dreschsel bottle that was maintained in a water bath at 50 deg C. The vapor stream was diluted with filtered conditioned air and passed into the inhalation chamber. The chamber was a 1.5 cubic meter in volume, constructed of stainless steel and glass and contained in which individually caged rats were confined to a single tier of cages occupying 0.5 cubic meters. Air exchange rate was 12 exchanges per hour.

Atmospheres were analyzed by infrared spectroscopy with a Foxboro Miran-1A Gas Analyzer using a continuous flow and automatic monitoring system. Calibration was done by injecting a known volume of liquid test material into the chamber with a precision syringe and running the chamber in a closed-loop mode until the reading stabilized.

Groups of 10 adult rats of each sex were exposed for 7 hours/day for 5 days to 0, 250 or 1000 ppm diglyme as a vapor. After their last exposure period, animals were injected ip with 3 mg colchicine/kg 2 hr prior to sacrifice. Three sampling times (6, 24, and 48 hr after the end of exposure) were used for the 1-day of exposure groups. The 5-day exposed groups were sacrificed 6 hours after completion of the final exposure.

After sacrifice, bone marrow was removed and fixed to a microscope slide prior to standard staining with Giemsa. Stained slides were labeled with numbers not directly correlated with the animal numbers; therefore, all assessments of metaphases were conducted "blind." Where possible, 50 metaphases per rat were scored.

Remark :

That the exposure levels were high enough for an adequate test of clastogenicity can be ascertained from the following information:

1.) Rats were severely sedated while in the chambers.

2.) The identical conditions and chambers were used to dose rate for the dominant lethal test. In this other test using the same equipment at the same dose, testes of male rats were severely affected as evidenced by

severe lack of reproductive function (ability to effectively cause pregnancy in an appropriate female) in high-dose exposed animals during the 4 to 8 week period after exposure.

3.) Using the same exposure concentrations and time, four of ten mice died as a result of only four exposures

Supporting a lack of clastogenicity is a study described in the same published report of the ability of diglyme to cause Unscheduled DNA Synthesis. In this in vitro study, concentrations of diglyme up to 19 mg/ml incubated with human embryonic intestinal fibroblasts (Flow 11,000 cells at passages 12 to 35) in the presence and absence of a S-9 mix to supply metabolic activation, did not induce the uptake of tritiated-thymidine using a standard procedure.

Also adding support is the information that the glycol ethers, as a class, lack significant genotoxic activity (ECETOC The toxicology of glycol ethers and its relevance to man. Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals, pp. 1-350 (Technical Report No . 64), 1995.

Result :

In neither the 5-day nor the single exposure test was there any good evidence for the induction of chromosomal damage, other than in the positive control groups. The only significant increases in aberrant cell frequency occurred in the low-dose male group 6 hr following a single exposure to the test material. Slides from male rats exposed to 250 ppm diglyme demonstrated a small increase in the frequency of total aberrations ($t = 2.216$, $p < 0.05$). These elevations were restricted to a single sex in each case and were not reproduced at the higher dose levels. It was concluded, therefore, that diglyme is not clastogenic

Test substance :

Diglyme, CASNO 111-96-6, obtained from Aldrich (batch 21150)

Conclusion :

Diglyme is not clastogenic to rats of either sex after high-dose administration by inhalation over 5 days

Reliability : (2) valid with restrictions

Published study with scientifically defensible design using appropriate dose levels.

Flag : Critical study for SIDS endpoint

28.12.2003 (15)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type	:	other: Dominent Lethal
Species	:	rat
Sex	:	
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	7h/day, 5 continuous days
Frequency of treatm.	:	daily for 5 days
Premating exposure period		
Male	:	fixed time exposure with recovery
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	250 or 1000 ppm
Control group	:	yes, concurrent vehicle

Method :

CD rats (Sprague-Dawley derived) were used and were obtained from Charles River (UK)

Test atmospheres were generated by bubbling dry oxygen-free nitrogen through diglyme contained in a Dreschsel bottle that was maintained in a water bath at 50 deg C. The vapor stream was diluted with filtered conditioned air and passed into the inhalation chamber. The chamber was a 1.5 cubic meter in volume, constructed of stainless steel and glass and contained in which individually caged rats were confined to a single tier of cages occupying 0.5 cubic meters. Air exchange rate was 12 exchanges per hour.

Atmospheres were analyzed by infrared spectroscopy with a Foxboro Miran-1A Gas Analyzer using a continuous flow and automatic monitoring system. Calibration was done by injecting a known volume of liquid test material into the chamber with a precision syringe and running the chamber in a closed-loop mode until the reading stabilized.

Groups of 10 adult male rats were exposed for 7 hours/day for 5 days to 0, 250 or 1000 ppm diglyme. After the exposure period they were serially mated with untreated virgin females, 2 females per male. The females were sacrificed 17 days after first being caged with exposed males and examined for evidence of pregnancy by the method of Bateman (Handbook of Mutagenicity Test Procedures ed. by BJ Kilbey et al., Elsevier, Amsterdam)

Result :

Exposure to 1000 ppm diglyme 7 hr/day for 5 days was associated with a significant reduction in the pregnancy frequency (females with implantations) starting with mating that occurred 4 weeks after dosing (week 4) where only 50% of mated females showed evidence of implantations. The pregnancy frequency was further reduced in week 5, 6 and 7 matings to about 10% of the mated females showing implantations when sacrificed 17 days after mating with males exposed to 1000 ppm diglyme. Air controls and male rats exposed to 250 ppm diglyme showed no changes in pregnancy frequency.

The number of corpora lutea per pregnancy, the live implants and the number of implantation sites were enumerated and manipulated using the Freeman-Tukey Poisson transformation and the Freeman-Tukey binomial transformation searching for evidence of early death that would be indicative of a dominants lethal effect. The mathematical transformations indicated some evidence of early deaths that could be indicative of a dominant lethal induction in exposed males. The results are given in the tables below:

##PREGNANCY FREQUENCY AND PERCENT

	DIGLYME CONCENTRATION					
WEEK	0		250 ppm		1000 ppm	
1	18/20	90%	19/20	95%	15/20	75%
2	19/20	95%	18/20	90%	19/20	95%
3	18/20	90%	18/19	95%	16/20	80%
4	19/20	95%	19/20	95%	10/20	50%
5	19/20	95%	18/20	90%	2/19	11%
6	20/20	100%	20/20	100%	2/20	10%
7	19/20	95%	20/20	100%	2/20	10%
8	19/20	95%	20/20	100%	8/20	40%
9	19/20	95%	19/20	95%	10/20	50%
10	18/20	90%	19/20	95%	17/20	85%

##TOTAL NUMBER CORPORA LUTEA PER PREGNANCY

	DIGLYME CONCENTRATION		
WEEK	0	250 ppm	1000 ppm
1	12.7 ±0.54	12.5 ±0.53	12.6 ±0.59
2	13.3 ±0.50	13.2 ±0.52	13.3 ±0.50
3	14.8 ±0.79	13.8 ±0.79	12.4 ±0.84*
4	12.2 ±0.54	12.5 ±0.54	11.1 ±0.74
5	13.8 ±0.55	12.6 ±0.57	12.5 ±1.71
6	13.4 ±0.55	12.3 ±0.55	7.0 ±1.75**
7	12.2 ±0.34	11.8 ±0.33	2.5 ±1.04***
8	11.8 ±0.62	11.9 ±0.60	11.9 ±0.96
9	13.7 ±0.57	13.2 ±0.57	12.0 ±0.79
10	12.9 ±0.51	12.3 ±0.50	12.6 ±0.52

##TOTAL IMPLANTATIONS PER PREGNANCY

	DIGLYME CONCENTRATION		
WEEK	0	250 ppm	1000 ppm
1	13.0 ±0.63	12.0 ±0.61	13.4 ±0.61
2	13.6 ±0.54	12.4 ±0.55	13.7 ±0.54
3	13.8 ±0.78	12.5 ±0.78	12.4 ±0.82
4	12.4 ±0.60	12.3 ±0.60	9.9 ±0.82*
5	13.5 ±0.53	13.1 ±0.55	11.0 ±1.64
6	13.0 ±0.47	13.6 ±0.47	2.5 ±1.49***
7	12.0 ±0.31	11.7 ±0.31	2.0 ±0.97***
8	12.1 ±0.68	12.3 ±0.67	11.8 ±1.06
9	11.6 ±0.63	12.4 ±0.63	10.4 ±0.87
10	12.3 ±0.50	12.1 ±0.49	11.5 ±0.52

##SUM OF LIVE IMPLANTS AND LATE DEATHS

	--------DIGLYME CONCENTRATION----------		
WEEK	0	250 ppm	1000 ppm
1	12.5 ±0.69	11.6 ±0.67	12.4 ±0.75
2	13.2 ±0.56	12.1 ±0.57	13.1 ±0.56
3	13.3 ±0.78	11.6 ±0.78	11.6 ±0.82
4	11.9 ±0.64	11.8 ±0.64	9.5 ±0.88*
5	13.3 ±0.54	12.4 ±0.55	9.5 ±1.65*
6	12.3 ±0.46	13.3 ±0.46	1.5 ±1.47***
7	11.3 ±0.33	11.5 ±0.32	2.0 ±1.01***
8	11.5 ±0.72	11.6 ±0.70	11.0 ±1.11
9	11.1 ±0.62	12.0 ±0.62	10.0 ±0.85
10	12.3 ±0.48	12.2 ±0.47	11.6 ±0.50

##EARLY DEATH FREQUENCY Freeman-Tukey Poisson Transformation

	--------DIGLYME CONCENTRATION----------		
WEEK	0	250 ppm	1000 ppm
1	1.63 ±0.209	1.52 ±0.204	2.00 ±0.229
2	1.52 ±0.186	1.43 ±0.192	1.86 ±0.186
3	1.67 ±0.262	1.96 ±0.262	1.63 ±0.278
4	1.64 ±0.196	1.60 ±0.196	1.50 ±0.269
5	1.30 ±0.188	1.82 ±0.193	2.78 ±0.579*
6	1.85 ±0.187	1.50 ±0.187	2.07 ±0.591
7	1.79 ±0.169	1.28 ±0.164	1.00 ±0.519
8	1.67 ±0.207	1.78 ±0.201	1.89 ±0.318
9	1.55 ±0.191	1.63 ±0.191	1.50 ±0.264
10	1.36 ±0.219	1.96 ±0.213	1.71 ±0.225

Test substance :

Diglyme, CASNO 111-96-6, obtained from Aldrich (batch 21150)

Conclusion :

Although there was some evidence for post-implantation loses that are suggestive of a dominant lethal effect, the result was confounded by the very low number of total implantation in those weeks. As there is an independent association between a low number of implantations and post implantation loss and as the numbers of poot-implantation losses were very small and only occurred in weeks where the number of total implantations was low, the results of the test are equivocal regarding a dominant lethal effect.

The authors concluded that the effect of diglyme "on male fertility and embryonic development are of much greater importance than genetic effects when setting tolerable limits"

Reliability : (2) valid with restrictions

Published study with robust design at appropriate dose levels.

Flag : Critical study for SIDS endpoint

28.12.2003 (15)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	mouse
Sex	:	female
Strain	:	CD-1
Route of admin.	:	gavage
Exposure period	:	gestation day 6-15
Frequency of treatm.	:	daily
Duration of test	:	to gd 17
Doses	:	0, 62.5, 125, 250 or 500 mg/kg-day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 500 mg/kg bw
NOAEL teratogen.	:	= 125 mg/kg bw
NOAEL Fetotoxicity	:	= 62.5 mg/kg bw
Result	:	Specific Developmental Toxin
Method	:	other: NTP
Year	:	
GLP	:	yes
Test substance	:	

Method :

Mice of the strain Crl:CD-1(ICR)Br were obtained form Charles River Laboratory (Kingston, NY) and maintained at 19.4 to 22.2 deg C and RH 40-76% on a 12 hour light/dark cycle with food and water ab libitum. Females were mated one to one with males of the same strain. Vaginal plug positive females (called gestational day zero when found) were randomly (stratified to keep mean body weights per group even) assigned to dose groups and group housed.

Diglyme () was administered to pregnant mice by oral gavage, using water as vehicle, on gestational days (gd) 6-15 in the morning between 8;30 and 10:30 AM at dose levels of 0, 62.5, 125, 250 or 500 mg/kg/day. These dose were selected on the basis of preliminary study on pregnant mice of the same strain. There were 20-24 confirmed pregnant mice per group, divided into two replicates at least 2 weeks apart. Mice were monitored daily during treatment for evidence of maternal toxicity.

OBSERVATIONS.
Mice were weighed alive on gd 0, 6 through 15, and immediately following sacrifice on gd 17. Dams were observed daily during treatment for clinical signs of toxicity. Maternal liver weights and gravid uterine weights were measured following sacrifice by cervical dislocation. Uterine contents were evaluated for the number of implantation sites, resorptions, late fetal deaths (i.e., fetuses with discernible digits and weighing greater than 0.3 g, but displaying no vital signs on gd 17) and live fetuses. When visible evidence of pregnancy was not observed, the uterus was stained with 10% ammonium sulfide to reveal possible early resorptions. Live fetuses were dissected from the uterus and anesthetized by using hypothermia. Each live fetus was weighed and examined for external morphological abnormalities. The viscera were then examined using a fresh tissue dissection technique. Half of the fetuses' heads were removed prior to dissection and the heads were fixed in Bouin's solution for free-hand sectioning and examination. All fetal carcasses were cleared and double stained with Alcian blue/Alizarin red S prior to examination for skeletal malformations.

STATISTICAL ANALYSES.

Analyses of data were carried out using the General Linear Model procedure in the SAS software library (SAS Institute, Inc., Cary NC). Prior to analysis, an arcsine-square root transformation was performed on all litter-derived percentage data. Dose-response relationships for selected measures were evaluated using a test for linear trend. Analysis of variance was employed to determine whether significant dose effects, replicate effects or dose X replicate interactions had occurred. When ANOVA revealed significant differences among groups, then Williams' multiple comparison test and Dunnett's test were used to compare diglyme-treated groups to the vehicle control group (using an alpha level = 0.05). Nominal scale measures were analyzed by a test for linear trend on proportions, and a XZ test for independence among treatment groups. When XZ revealed significant ($p < 0.05$) differences among groups, then a one-tailed Fisher exact probability test (alpha level = 0.05) was used for pairwise comparisons between each treated group and the vehicle control group.

Remark :

This result is also supported by an inhalation study of pregnant rats indicating diglyme is a developmental toxin below maternally toxic doses (i, ii) and a gavage study in rabbits indicating developmental toxicity in the rabbit (iii, iv)

i.) Driscoll CD, Valentine R, Staples RE, Chromey NC, Kennedy GL Jr Developmental toxicity of diglyme by inhalation in the rat. Drug and chemical toxicology, 21(2):119-136 (1998).

ii.) DuPont Teratogenicity study of diglyme in the rat . Newark, NJ, E.I. Du Pont de Nemours & Co., 289 pp .1988

iii.) Schwetz BA, Price CJ, George JD, Kimmel CA, Morrissey RE, Marr MC The developmental toxicity of diethylene and triethylene glycol dimethyl ethers in rabbits. Fundamental and applied toxicology, 19(2):238-245 (1992)

iv.) NTP Teratologic evaluation of diethylene glycol dimethyl ether (CAS No. 111-96-6) administered to New Zealand White rabbits on gestation days 6 through 19. Research Triangle Park, NC, National Institute of Environmental Health Sciences, National Toxicology Program NTP-87-108; PB 87-209532, (1987)

Result :

Diglyme-treated dams did not exhibit treatment-related clinical signs, death, or differences from control in corrected maternal body weight gain (i.e., gestational weight gain minus gravid uterine weight) or relative maternal liver weight. Maternal intact body weight (including gravid uterine weight) was reduced in groups dosed with 250 and 500 mg/kg-day on gd 15 and 17, as was maternal weight gain during treatment and gestation. Gravid uterine weight in diglyme-treated groups was significantly reduced at all doses in a dose-related manner. There were dose-related and significant increases at 250 and 500 mg/kg/day in the percentage of nonlive implants per litter (4.88%, 8.41%, 7.05%, 12.02% and 50.41% in the vehicle control through high-dose groups, respectively), as well as the percentage of adversely affected implants per litter (5.25%, 8.41%, 9.35%, 32.29% and

96.93% nonlive or malformed in the vehicle control through high-dose groups, respectively).

The mean live litter size was significantly less than control at 500 mg/kg/day and was marginally reduced at all lower dose levels. The mean fetal body weight per litter was reduced at and above 125 mg diglyme/kg-day. Anatomical malformations displayed a dose-dependent trend toward an increased percentage of malformed live fetuses per litter, and the difference from control was statistically significant in the 250 and 500 mg groups. The mean percent malformed live fetuses per litter was 0.37%, 0.00%, 2.47%, 23.86% and 95.82% in the vehicle through high-dose groups, respectively. The proportion of litters with gross, visceral or skeletal malformations was increased at the high dose, and the proportion of litters with gross or skeletal malformations was increased in the 250 mg/kg-day group. The types of malformations observed were diverse. Major malformations affected primarily development of the neural tube, limbs and digits, craniofacial structures, abdominal wall, cardiovascular system, urogenital organs, and both the axial and appendicular skeleton. The two most frequently observed malformations were fused ribs in 74% of high dose fetuses and exencephaly in 54% of high dose fetuses.

Exposure of pregnant CD-1 mice to diglyme throughout the period major organogenesis produced no notable evidence of maternal toxicity. The lowest dose level, 62.5 mg/kg/day, appeared to be a no observed effect level for indices of fetal development. At higher doses, diglyme produced adverse effects upon fetal growth (greater than or equal to 125 mg/kg/day), fetal viability (greater than or equal to 250 mg/kg/day), and fetal morphological development (greater than or equal to 250 mg/kg/day). At the highest dose, all 23 litters contained at least one malformed fetus compared to 1 of 21 control litters, and 94% of the high-dose fetuses were malformed compared to 0.35% of the fetuses in the control group. Thus, diglyme administration by gavage to the pregnant mouse represents a risk to the embryo or fetus at dose levels that did not cause observable toxicity to the maternal organism.

MATERNAL PARAMETERS

		-------Diglyme mg/kg----------			
	0	62.5	125	250	500
Treated dams	28	28	29	28	28
Preg at sac	21	20	24	23	23
Mat BW gd-0	30.67	31.03	30.75	30.5	30.46
Mat BW gd-17	56.3	53.41	54.5	52.17**	47.09**
Maternal wt gain					
- gestation	25.63	22.38	23.75	21.67**	16.64**
- treatment	15.32	13.3	14.06	12.98**	10.03**
- corrected	6.28	6	6.4	5.72	6.14
Uterus wt	19.35	16.38*	17.35*	15.96**	10.50**
Mat Liver wt	3.08	2.94	2.99	2.89	2.65**
Relative mat liver					
Weight % of body	5.48	5.52	5.49	5.54	5.62

All weights in grams * = p < 0.05, ** = p < 0.01

LITTER/PUP PARAMETERS

	-------Diglyme mg/kg----------				
	0	62.5	125	250	500
Litters Examined	21	20	24	23	23
Implant site/lit	14.10	12.10	13.00	13.00	12.39
% Resorp/litter	4.88	7.37	6.70	9.94	45.63**
% Litters with	47.6	55.0	62.5	60.9	100.0**
Post Implantation loss (%/liter)	4.88	8.41	7.05	12.02*	50.41**
- litters with %	47.6	60.0	62.5	69.6	100.0**
% Litters with adversely aff imp	52.4	60.0	70.8	95.7*	100.0**
Live pups/litter	13.43	11.24*	12.08*	11.35**	6.13**
% males/litter	47.19	49.23	44.79	51.69	52.60
Mean fetal BW/lit	1.003	0.972	0.912**	0.796**	0.554**
Live fetuses % Malformed/litter	0.37	0.00	2.47	23.86**	95.82**
Lit with malformed Pups (%)	4.76	0.00	25.0	82.61**	100.0**

	-------Diglyme mg/kg----------				
	0	62.5	125	250	500
Malformed fetuses /total examined	1/282	0/225	7/290	59/261**	132/141**
Litt with mald	1/21	0/20	6/24	19/23**	23/23**
Pups with					
- External malf	0	0	3	7	93
- Visceral malf	1	0	1	2	28
- Skeletal malf	0	0	3	52	112

* = p < 0.05, ** = p < 0.01

Test substance :

Diglyme, CASNO 111-96-6 >99% pure

Conclusion :

Diglyme is considered a specific developmental toxin in the mouse producing adverse effects on the conceptus at dose levels not associated with maternal toxicity.
The maternal NOAEL is considered 500 mg/kg-day
The fetal NOAEL is considered 62.5 mg/kg-day

Reliability : (1) valid without restriction

Published GLP study with robust design at appropriate dose levels. Full documentation is available.

Flag : Critical study for SIDS endpoint

28.12.2003 (17)

(1) Calculated from the experimental rate constant published by Dagaut P, Wallington IJ, Liu R, Kurylo MJ (1988) 22nd International Symposium on Combustion, Seattle, WA; as cited in Atkinson R (1989) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. Journal of Physical and Chemical Reference Data, 1:143. as cited in Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

(2) Calculation performed by Toxicology and Regulatory Affairs, December 2003

(3) Data found on NTP public database at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

(4) Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989 as cited in National Library of Medicine Hazardous Substance Data Base, Record #72, Last Revision Date: 20030305

(5) DuPont Chemical, Haskell Laboratories report 51-73. Initial Submission: Acute Oral Test of Ether,

(6) Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society. 1995

(7) Harris, JC. Rate of hydrolysis. In: Lyman WJ, Reehl WF, Rosenblatt DH, eds. Handbook of chemical property estimation methods: Environmental behavior of organic compounds. New York, NY, McGraw-Hill Book Company, pp. 7-1-7-48. (1990)

(8) Hoechst (1979a) Inhalationstoxizität im Zeitsättigungstest von Diethylenglykol-dimethylether an männlichen und weiblichen SPF-Wistar-Ratten. Frankfurt am Main, Hoechst AG, 10 pp . (Report 488/79; unpublished) as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

(9) Hoechst (1989) Prüfberichte: ökologische Untersuchungen. Frankfurt am Main, Hoechst AG, 4 pp. (unpublished report). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002

(10) Hoechst (1994) Prüfung der toxischen Wirkung von Diethylenglykoldimethylether auf Kleinkrebse (Daphnia magna). Hoechst AG, Frankfurt am Main (unpublished report) .as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

(11) Hoechst (1995) Prüfung der Schadwirkung gegenüber Algen (Algentoxizität) von Diethylenglykoldimethylether. Hoechst AG, Frankfurt am Main (unpublished report). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

(12) Hoechst Abwasserbiologische Untersuchung von Dialkylglykoläthern auf die Goldorfe (Leuciscus idus). Report Nr.79.0555 (1979) as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002 and using data found in the ECB IUCLID 2000 document for diglyme.

(13) Hoechst AG (1989): Unveroeffentlichte Untersuchung (V-89-903-A) as cited in European Chemicals Bureau, IUCLID 2000 for CASNO 111-96-6

(14) Hoechst AG : Unveroeffentlichte Untersuchung (79.0488) 1979 as cited in ECB IUCLID-2000 for diglyme.

(15) McGregor DB, Willins MJ, McDonald D, Holmstrom M, McDonald D, Niemeier RW Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl)ether. Toxicology and applied pharmacology, 70:303-316 (1983)

(16) O'Neil, MJ (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001

(17) Teratologic Evaluation of Diethylene Glycol Dimethyl Ether (CAS NO. 111-96-6) Administered to CD-1® Mice on Gestational Days 6 through 15, NTP Study TER85061 Report Date: August 30, 1985
NTIS#PB86-135233

Also published as:
Price CJ, Kimmel CA, George JD, Marr MC The developmental toxicity of diethylene glycol dimethyl ether in mice. Fundamental and applied toxicology, 8:115-126 (1987).

(18) Teratologic evaluation of diethylene glycol dimethyl ether (CAS No. 111-96-6) administered to New Zealand White rabbits on gestation days 6 through 19. (1987) Research Triangle Park, NC, National Institute of Environmental Health Sciences, National Toxicology Program (NTP-87-108; PB 87-209532). as cited in: Concise International Chemical Assessment Document 41, Diethylene Glycol Dimethyl Ether. International Program on Chemical Safety, World Health Organization Geneva, 2002.

(19) Valentine R, O'Neill AJ, Lee KP, Kennedy GL Jr. Subchronic inhalation toxicity of diglyme. Food Chem Toxicol. 37:75-86 (1999)